Testosterone
and associated biological and psychological factors
in alcohol and opiate addiction

INAUGURALDISSERTATION
zur Erlangung des Grades einer Doktorin
der Humanbiologie
-Doctor rerum biologicarum humanarum-
(Dr. rer. biol. hum.)

vorgelegt von

Katrin Stange
aus Hannover

Hannover 2017
Angenommen durch den Senat: 18.12.2017

Präsident: Prof. Dr. med. Christopher Baum

Wissenschaftliche Betreuung: Prof.’in Dr. med. Annemarie Heberlein

Wissenschaftliche Zweitbetreuung: Prof.’in Dr. med. Dr. phil. Astrid Müller

1. Referent: Prof.’in Dr. med. Annemarie Heberlein
2. Referent: Prof.’in Dr. med. Dr. phil. Astrid Müller
3. Referent: PD Dr. rer. nat. Gregor Szycik

Tag der mündlichen Prüfung: 18.12.2017

Prüfungsausschuss

Vorsitz: Prof.’in Dr. med. Dr. phil. Astrid Müller
1. Prüfer: Prof.’in Dr. med. Annemarie Heberlein
2. Prüfer: Prof.’in Dr. med. Dr. phil. Astrid Müller
3. Prüfer: PD Dr. rer. nat. Gregor Szycik
Acknowledgements

I would like to express my sincerest gratitude to my advisors Prof. Dr. Annemarie Heberlein and Prof. Dr. Dr. Astrid Müller for encouraging my research and for the continuous support, motivation, and guidance that have helped me to successfully conclude my dissertation. Furthermore, I would like to sincerely thank all three reviewers for the evaluation of my dissertation.

I am very grateful to Thilo Janssen and Kerstin Stange, who have patiently proofread the manuscript, and to my whole family for supporting me throughout the process of writing this dissertation.

I would like to cordially thank my co-authors for the excellent cooperation during the work on the two articles that constitute the basis of this dissertation. Finally, I would like to especially thank all participants of the two studies without whose willingness to take part in scientific research my dissertation would not have been possible.
Table of contents

Summary 7
Zusammenfassung 10

1. Introduction 13
1.1 Biopsychosocial models of addiction 13
1.2 Testosterone and addiction 13
1.3 BDNF and addiction 16
1.4 Interplay of testosterone and BDNF 17
1.5 Empathic abilities and addiction 18
1.6 Association of testosterone with empathic abilities 20
1.7 Aims of the two studies 22

2. Study 1 – Association of testosterone and BDNF serum levels with craving during alcohol withdrawal 24
2.1 Methods 24
2.1.1 Sample 24
2.1.2 Measures and procedure 24
2.1.3 Data analysis 25
2.2 Results 26
2.2.1 Group differences regarding testosterone between the patients’ group and the control group 26
2.2.2 Group differences regarding testosterone between subgroup A and subgroup B 26
2.2.3 Alterations of testosterone levels during alcohol withdrawal in the patients’ group 27
2.2.4 Association of testosterone with BDNF and cortisol among the patients’ group 27
2.2.5 Association of testosterone with severity of alcohol addiction 28
2.2.6 Association of testosterone with alcohol craving 28
2.2.7 Associations of testosterone, BDNF, and craving in subgroup A 28

3. Study 2 – Positive association of personal distress with testosterone in opiate-addicted patients 29
3.1 Methods 29
3.1.1 Sample 29
3.1.2 Measures and procedure 29
3.1.3 Data analysis 30
3.2 Results 32
3.2.1 Preliminary analyses 32
3.2.2 Group differences regarding testosterone between the patients’ group and the control group

3.2.3 Group differences regarding empathy between the patients’ group and the control group

3.2.4 Association of testosterone with empathy among the patients’ group

3.2.5 Complementary results

4. Discussion

4.1 Testosterone during alcohol and opiate withdrawal

4.2 Interplay of testosterone and BDNF in alcohol addiction

4.3 Cognitive and emotional empathy in opiate addiction

4.4 Positive association of testosterone with personal distress in opiate addiction

4.5 Limitations

4.6 Clinical implications

4.7 Further research

5. References

Annex I

Paper study 1: Association of testosterone and BDNF serum levels with craving during alcohol withdrawal

Paper study 2: Positive association of personal distress with testosterone in opiate-addicted patients

Annex II

Lebenslauf und wissenschaftliche Veröffentlichungen

Erklärung über die selbstständige Verfassung

Erklärung zur Verfügbarkeit der Originaldaten

Einverständniserklärung zur Plagiatsüberprüfung
List of tables

Table 1: Sample characteristics of the alcohol-addicted patients and healthy controls 26
Table 2: Group comparisons regarding testosterone, BDNF, and cortisol serum levels between the group of alcohol-addicted patients and the healthy control group 27
Table 3: Sample characteristics of the opiate-addicted patients and healthy controls 31
Table 4: Group comparisons regarding testosterone levels and empathy scores between the group of opiate-addicted patients and the healthy control group 32

List of figures

Figure 1: Testosterone serum levels during alcohol withdrawal (day 1, day 7, day 14) in the group of alcohol-addicted patients compared to the testosterone levels of the healthy control group (C) Figure derived from study 1 (see Annex I). 27
Summary

Katrin Stange – Testosterone and associated biological and psychological factors in alcohol and opiate addiction

According to biopsychosocial models a complex interplay of biological, psychological, and social factors influences the development and maintenance of a substance addiction. One line of research on biological factors focuses on the sex hormone testosterone. Studies on chronic alcohol consumption or opiate use typically found lower testosterone levels in patients compared to healthy controls. But when examining testosterone levels during or after withdrawal, alcohol-addicted patients were found to display higher levels than controls. Another line of research focuses on the brain-derived neurotrophic factor (BDNF) which has been reported to be associated with substance use disorders. Testosterone and BDNF interact in various contexts and a possible interplay in regard to substance addiction may be assumed. In this respect, the activity of the hypothalamic-pituitary-adrenal (HPA) axis may play an important role, as the HPA axis and the hypothalamic-pituitary-gonadal (HPG) axis have been shown to interact with each other and both seem to modulate not only BDNF activity but also impulsive behavior. With respect to psychological factors, previous clinical studies reported that substance addictions are associated with sociocognitive deficits such as impairments regarding empathic abilities. As testosterone is linked with aggression and dominance behavior, it may be a possible physiological correlate and biomarker of these impairments. Evidence supporting this assumption stems from studies on the possible role of fetal testosterone regarding social cognition deficits and testosterone administration studies with healthy subjects reporting empathy impairments after the application of the hormone.

In the two studies presented, testosterone levels in addicted patients during withdrawal were compared to the levels of healthy control subjects. Furthermore, possible associations between testosterone and other biological as well as psychological factors were examined. In study 1, testosterone levels in alcohol-addicted patients during withdrawal were compared to the levels of healthy controls and possible alterations of testosterone levels during the course of alcohol withdrawal were examined. Furthermore, associations between...
testosterone, BDNF, and the symptomatology of alcohol withdrawal were investigated, as well as associations between these factors in subgroups of patients with high versus low HPA activity. The sample contained 99 male alcohol-addicted patients (age mean 42.22 years, SD 7.83) and 17 healthy male controls (age mean 44.41 years, SD 9.63). BDNF, testosterone, and cortisol serum levels were assessed on day 1, day 7, and day 14 of alcohol withdrawal in the patient’s group. Alcohol withdrawal symptomatology was measured via self-report. Higher testosterone levels were found in the patients’ group compared to the control group (day 1: $U = -3.652, p < 0.001$, day 7: $U = 3.238, p = 0.001$, day 14: $U = 3.019, p = 0.003$). There was a decrease of testosterone levels among the patients’ group during alcohol withdrawal ($\chi^2 = 32.23, p < 0.001$). The decrease of testosterone levels in the patients’ group during early withdrawal was negatively associated with the BDNF levels at the beginning of withdrawal ($\rho = -0.268, p = 0.008$) and positively associated with alcohol craving in the middle of the examined withdrawal period ($\rho = 0.248, p = 0.014$). In the subgroup of patients with higher cortisol serum levels, mirroring high activity of the HPA axis, the decrease of testosterone levels throughout alcohol withdrawal was negatively associated with BDNF levels ($\rho = -0.369, p = 0.013$) and positively associated with craving in the middle of the withdrawal period ($\rho = 0.417, p = 0.004$). In this subgroup, the BDNF serum levels were positively associated with alcohol craving at the beginning of withdrawal ($\rho = 0.429, p = 0.003$).

In study 2, testosterone levels in opiate-addicted patients during early withdrawal were compared to the levels of healthy controls. Furthermore, empathic abilities in opiate-addicted patients were compared to those of controls to examine whether patients show impairments in cognitive and emotional empathy in comparison to healthy controls, and possible associations between testosterone levels and empathic abilities among the patients’ group were investigated. The sample contained 27 opiate-addicted, diacetylmorphine-maintained patients (21 males, age mean 41.67 years, SD 8.814) and 31 healthy controls (23 males, age mean 40.77 years, SD 8.401) matched in age, sex, and educational level. Cognitive (perspective taking) and emotional empathy (empathic concern, personal distress) were measured via self-report and salivary testosterone levels were assessed. Higher personal distress scores ($t = 3.100, p = 0.002, d = 0.817$) and higher testosterone levels ($t = 3.955, p < 0.001, d = 1.093$) were found in the patients’ group compared to controls. Moreover, a positive correlation between testosterone and personal distress among the patients’ group was found ($r = 0.399, p = 0.039$).
The results of both studies support the assumption that the sex hormone testosterone plays an important role in regard to substance addiction. Consistent with previous research, higher testosterone levels in addicted patients during withdrawal in comparison to the testosterone levels of controls were found both in alcohol and opiate addiction. The results of study 1 indicate that the testosterone levels of alcohol-addicted patients are especially high during early withdrawal and decrease subsequently. Moreover, a positive association of the decrease of testosterone levels and craving was found among the group of alcohol-addicted patients. The reported findings suggest that testosterone and the HPG axis can be assumed new targets for the treatment of opiate and alcohol addiction. Taking into account the results of previous studies, it can be hypothesized that high testosterone levels during early withdrawal might be considered a marker for severe addiction. Furthermore, the results of study 1 support the assumption that there is a relevant association between testosterone and BDNF in regard to alcohol addiction and indicate that alcohol craving during alcohol withdrawal may be explained by a complex interplay of testosterone, BDNF and HPA axis activity. The results of study 2 indicate that opiate-addicted patients display specific empathy impairments, namely high personal distress. This impairment may play an important part in the maintenance of opiate addiction, because opiates are able to alleviate the negative emotional states habitually evoked by high personal distress. The observed association of personal distress and testosterone in opiate-addicted patients points toward testosterone as a possible biomarker of empathy impairments in opiate-addicted patients, which has not been investigated before.
Zusammenfassung
Katrin Stange – Testosteron und assoziierte biologische und psychologische Faktoren bei Alkohol- und Opiatabhängigkeit


gesunden Kontrollen verglichen und mögliche Veränderungen der Testosteron-Werte im Verlauf des Entzugs geprüft. Weiterhin wurden Zusammenhänge zwischen Testosteron, BDNF und der Symptomatik des Alkoholentzugs untersucht sowie Zusammenhänge zwischen diesen Faktoren in Subgruppen von Patienten mit hoher im Gegensatz zu niedriger HPA-Aktivität. Das Sample bestand aus 99 männlichen alkoholabhängigen Patienten (Altersdurchschnitt 42,22 Jahre, SD 7,83) und 17 gesunden männlichen Kontrollen (Altersdurchschnitt 44,41 Jahre, SD 9,63). Die Serumwerte von BDNF, Testosteron und Kortisol wurden in der Patientengruppe an den Tagen 1, 7 und 14 des Alkoholentzugs erhoben. Die Entzugssymptomatik wurde fragebogengestützt erfasst. In der Patientengruppe wurden im Vergleich zur Kontrollgruppe höhere Testosteron-Werte gefunden (Tag 1: U = -3,652, p < 0,001, Tag 7: U = 3,238, p = 0,001, Tag 14: U = 3,019, p = 0,003). Es zeigte sich ein Abfall der Testosteron-Werte der Patientengruppe während des Alkoholentzugs (χ² = 32,23, p < 0,001). Der Abfall im frühen Entzug wies eine negative Assoziation mit den BDNF-Werten zu Beginn des Entzugs (rho = -0,268, p = 0,008) und eine positive Assoziation mit Craving in der Mitte des Entzugsverlaufs (rho = 0,248, p = 0,014) auf. In der untersuchten Subgruppe von Patienten mit höheren Kortisol-Werten – dies spiegelt eine hohe Aktivität der HPA-Achse wider – war der Abfall der Testosteron-Werte während des Entzugs negativ assoziiert mit den BDNF-Werten (rho = -0,369, p = 0,013) und positiv assoziiert mit Craving in der Mitte des Entzugsverlaufs (rho = 0,417, p = 0,004). In dieser Subgruppe korrelierten die BDNF-Werte positiv mit Craving zu Beginn des Entzugs (rho = 0,429, p = 0,003).

erhoben. In der Patientengruppe wurden im Vergleich zur Kontrollgruppe höhere Personal-Distress-Werte (t = 3,100, p = 0,002, d = 0,817) und höhere Testosteron-Werte gefunden (t = 3,955, p < 0,001, d = 1,093). Des Weiteren zeigte sich in der Patientengruppe eine positive Korrelation zwischen Testosteron und Personal Distress (r = 0,399, p = 0,039).


Die Ergebnisse von Studie 2 weisen auf spezifische Beeinträchtigungen in der Empathiefähigkeit bei opiatabhängigen Patienten hin, und zwar erhöhte Personal-Distress-Werte. Dies könnte eine wichtige Rolle in der Aufrechterhaltung der Opiatabhängigkeit spielen, da Opiate die negativen emotionalen Zustände lindern können, die durch hohen Personal Distress regelmäßig ausgelöst werden. Der beobachtete Zusammenhang zwischen Personal Distress und Testosteron bei opiatabhängigen Patienten deutet darauf hin, dass Testosteron ein Biomarker für die Empathiedefizite bei opiatabhängigen Patienten sein könnte, was bisher noch nicht untersucht wurde.
1. Introduction

Substance addictions are severe psychiatric disorders. For the affected individual, they significantly reduce quality of life and may entail disability or premature death, including mortality due to serious health issues, overdose-related deaths, and suicides (1). The economic burden for society, i.e. costs for medical care, productivity losses, crime, and social welfare, is high (2), making substance addictions a major public health problem. This underscores the importance of investment in research, prevention, and treatment of these serious disorders. Two major substance addictions are alcoholism and opiate addiction. According to data from 2015, 6.2 % of adults aged 18 or older in the USA were positive for alcohol use disorder in the past year, 0.2 % were positive for heroin use disorder (3).

1.1 Biopsychosocial models of addiction

Regarding the etiology and maintenance of substance use disorders, biopsychosocial models were postulated integrating multiple risk and protective factors. These models suggest a complex interaction of genetics, neurobiology, psychological factors, and environmental characteristics such as influences of peer groups, family, socioeconomic background, substance availability or cultural norms (4, 5, 6). The contributing factors are assumed to differentially influence the likelihood of initial substance use, the development and maintenance of an addiction, and probability of relapse. It is crucial to investigate the interplay of biological, psychological, and social factors on every level. Identifying the factors involved in the initiation of substance use and the development of substance use disorders can help to detect groups or individuals at risk and to establish interventions reducing the influence of risk factors and strengthening the effect of protective factors. Identifying the factors involved in the maintenance of substance use disorders can help to find novel approaches to pharmacological or psychotherapeutic treatment.

1.2 Testosterone and addiction

A promising line of research on biological factors involved in substance use disorders concentrates on sex hormones. Epidemiological data show that substance use disorders such as alcoholism and opiate addiction have a higher prevalence in males than in females (6). For opiate-using individuals, the ratio of male to female is reportedly as high as four to one (7).
Regarding alcohol use, data from a multinational project show that – although they differ in size – sex differences are universal across countries (8). Men on average exceed women in alcohol consumption per se and are more likely to be high-volume drinkers. Drinking men are more likely than drinking women to be high-frequency drinkers and to engage in heavy episodic drinking. Lifetime abstention from alcohol, on the other hand, is more prevalent among women. There are sex differences regarding all phases of substance use disorders, i.e. initiation of substance use, escalation, maintenance of the addiction, withdrawal, and relapse following abstinence (9, 10). The general pattern of these sex differences is universal for all substances (9). As sex hormones are involved in central nervous system regulation, they qualify as a possible biological basis for these sexual dimorphisms and it is assumed that they play an important role in the neurobiology of substance addictions (11, 12).

Men display higher levels in the sex hormone testosterone than women (13, 14). Considering the sex differences in prevalence rates of substance use disorders, this suggests a possible positive association between testosterone and substance addiction. Evidence supporting this assumption stems from preclinical as well as clinical research. With respect to alcohol, preclinical studies reported higher basal testosterone levels in alcohol-preferring rats in comparison to non-preferring rat lines (15) and a positive association between testosterone release and alcohol consumption in alcohol-preferring rats (16). Testosterone seems to have an effect on alcohol consumption: For example, Braams and colleagues reported that high testosterone levels in adolescents predicted a high amount of alcohol consumed in adulthood (17). In turn, alcohol consumption affects testosterone secretion. Therefore, the relationship between testosterone and alcohol use is bidirectional; once alcohol is consumed, it may subsequently influence the way that sex hormone activity affects alcohol intake (12). Ethanol was found to be directly linked to the biosynthesis of testosterone and to increase testosterone levels in male rats (18). Consistently, Sarkola and Eriksson reported that in male human subjects, testosterone levels increased two hours after a low dose (0.5g/kg) of ethanol (19). In premenopausal women, total testosterone levels were significantly higher 45 minutes and 90 minutes after ethanol ingestion than in the placebo condition (20). These effects are dose-dependent, as the intake of higher doses of ethanol decreases testosterone levels (21). Likewise, chronic alcohol consumption was typically reported to have a suppressive effect on testosterone levels. Alcohol-addicted patients displayed lower testosterone levels compared to healthy controls (22, 23). During
and after alcohol withdrawal, testosterone levels were found to be increased in addicted patients. Walter and colleagues reported that the testosterone levels of alcohol-addicted men were significantly higher after six weeks of abstinence compared to the testosterone levels of controls (24). Hasselblatt and colleagues found higher testosterone levels in alcohol-addicted patients over a period of 18 weeks of controlled abstinence compared to controls (25).

Similar results regarding a suppressive effect of chronic substance use on testosterone levels were reported with respect to opiate addiction. Studies on chronic opiate use typically found lower testosterone levels in patients compared to controls (26, 27). There is not much research yet on testosterone levels during opiate withdrawal but effects similar to those reported for alcohol withdrawal could be hypothesized. Preclinical results suggest that testosterone is involved in opiate withdrawal symptomatology. For example, testosterone was associated with opiate withdrawal syndrome in rats (28).

Because testosterone and impulsivity are linked (29, 30), previous research on impulsivity may add further evidence underlining the relevance of testosterone for substance addiction. The association between impulsivity and substance use disorders is well documented, in particular regarding alcohol consumption and opiate use (31, 32, 33). Heightened trait impulsivity has been identified as a risk factor for the development of substance use disorders (34, 35, 36). Sher and colleagues reported that the trait of behavioral disinhibition, which reflects an aspect of impulsivity, predicted substance abuse six years later (37). High impulsivity was also suggested to be a cognitive marker for substance addiction that does not recover after abstinence (38). While high trait impulsivity is viewed as a risk factor for the development of a substance use disorder, prolonged substance use may in turn have the effect of an increase in impulsivity, which can further facilitate substance use (39).

Therefore, high impulsivity seems to be a factor relevant for the etiology and maintenance of addiction. During adolescence, when the role of sex hormones becomes more important, impulsivity increases, especially in boys (40). Adolescence is the typical time of onset for substance use and addiction as well (6, 40). Externalizing behaviors such as substance abuse and conduct disorder highly co-occur in adolescents and are strongly associated with impulsivity (41). Externalizing psychopathology is more prevalent among adolescent boys than among adolescent girls (42) and increases at a higher rate among men in young adulthood compared with women of the same age group, widening the gap between the
sexes from a modest to a large one (43). Furthermore, when controlling for age, advanced pubertal maturation was found to be linked to increased alcohol use in adolescents and higher testosterone levels were associated with the onset of alcohol use in boys (44). These findings support the assumption that testosterone is an important target for advancing our knowledge on the etiology and maintenance of substance addiction.

1.3 BDNF and addiction

Another interesting line of research on biological factors associated with substance use disorders focuses on the brain-derived neurotrophic factor (BDNF). BDNF is a member of the neurotrophic factor family and the most prevalent growth factor in the central nervous system. It is essential for the development and plasticity of the brain and plays a crucial role in the survival and differentiation of neurons (45, 46, 47). Altered BDNF production and secretion were reported to be linked with the development of cognitive deficits associated with aging and neurodegenerative diseases such as Alzheimer’s and Parkinson’s diseases (48). BDNF also seems to play an important role in the pathophysiology of several psychiatric disorders such as depression (49) and anxiety disorders (50).

A possible involvement of BDNF in substance use disorders has been suggested (47, 51, 52). Studies regarding BDNF serum levels in alcohol-addicted patients typically found lower levels in patients compared to controls (53, 54). Joe and colleagues reported that in a group of alcohol-addicted patients, the subgroup of patients with a positive family history of alcohol addiction had lower BDNF levels compared with the subgroup of patients with a negative family history of alcohol addiction (51). A positive association of BDNF levels and severity of alcohol withdrawal was found (55). After alcohol detoxification treatment, BDNF serum levels were reported to be increased (54, 55). For example, BDNF serum levels of alcohol-addicted patients both with and without symptoms of delirium tremens were increased significantly one week after alcohol withdrawal compared to levels on the first day of withdrawal. The group of patients without symptoms of delirium tremens displayed levels comparable to those of healthy controls one week after withdrawal, while the group of patients with delirium tremens still showed lower levels compared to controls. These results suggest that patients with more deficient BDNF expression are at a higher risk of developing delirium tremens (54).
Furthermore, BDNF serum levels may be a possible marker for alcohol relapse probability. Costa and colleagues examined the BDNF serum levels in a group of alcohol-addicted patients at the time of hospitalization for alcohol withdrawal and six months later and compared them to the levels of controls. They found that the BDNF serum levels of the subgroup of patients who remained abstinent during the six months after detoxification increased to a greater extent compared with those of the relapsing subgroup and were higher compared with the serum levels of controls (56). These results suggest that high BDNF serum levels after detoxification may reduce the risk of relapse in alcohol-addicted patients. Moreover, Heberlein and colleagues found evidence for a possible protective effect of high BDNF expression regarding relapse in alcohol-addicted patients. Mean methylation of the BDNF promoter was higher in a group of alcohol-addicted patients compared to age-matched healthy controls and decreased significantly during alcohol withdrawal. Associations of mean methylation of the BDNF promoter with depressive and anxious symptoms among the group of alcohol-addicted patients were reported. Methylation rates of the subgroup of patients abstaining longer before relapse were higher on day 14 of alcohol withdrawal in comparison to the subgroup of patients abstaining shorter (57).

With respect to opiate addiction, similar findings pointing to a high relevance of BDNF have been reported (47). BDNF serum levels were found to be lower in opiate-addicted patients compared to healthy controls (58, 59, 60) and increased after a longer period of abstinence (58). Heberlein and colleagues found a positive association of BDNF serum levels and heroin craving in diacetylmorphine-maintained opiate-addicted patients (61).

1.4 Interplay of testosterone and BDNF

Sex hormones such as testosterone and estrogen were reported to modulate BDNF expression and function (62, 63). While previous preclinical studies have suggested possible ways of interaction between sex hormones and BDNF, the interplay of these factors is not fully understood.

In the adolescent gonadectomized monkey and rat, gene expression of BDNF transcripts in frontal cortices was found to be reduced; testosterone replacement prevented this effect (64). Testosterone seems to affect hippocampal function and structure via modulation of BDNF (65, 66). For example, in male orchidectomized rats, synaptic transmission and excitability in the mossy fiber system was reported to be enhanced; an effect that was
shown to be reversed by testosterone replacement. Atwi and colleagues concluded that testosterone tonically suppresses mossy fiber BDNF levels (66). Testosterone was found to improve functional recovery after stroke in the male rat, possibly mediated by promoting BDNF levels (67). Testosterone and BDNF interactions are also involved in the development and maintenance of several features of neuromuscular systems (68). For instance, testosterone was found to regulate BDNF in spinal motoneurons and their corresponding target musculature (69).

A relevant factor to consider regarding the possible interplay of testosterone and BDNF with respect to substance use disorders is the activity of the hypothalamic-pituitary-adrenal (HPA) axis. This is because not only the hypothalamic-pituitary-gonadal (HPG) axis but also the HPA axis seems to modulate BDNF activity (70). Alterations in HPA axis activity have been repeatedly reported to be associated with substance use disorders (71, 72, 73). The HPA axis and the HPG axis have been shown to interact with each other (74, 75, 76). Moreover, this interaction appears to be functionally relevant in regard to impulsivity and substance addiction. Testosterone and cortisol seem to act jointly to regulate impulsive behavior and aggression (77, 78, 79). The interplay of the HPA and the HPG axis has been shown to play a role with respect to alcohol craving (80) and voluntary alcohol consumption in rats (81). Previous research also points toward an interaction of stress hormones and BDNF (82, 83, 84). While stress hormones seem to affect BDNF expression and signaling (84, 85), BDNF in turn was suggested to be a stress-responsive intercellular messenger (86) modulating the activation of the HPA axis (87). Therefore, the question arises whether there is a relevant interplay of BDNF, testosterone and HPA axis activity with respect to substance use disorders.

1.5 Empathic abilities and addiction

In addition to studies on biological factors it is equally important to investigate psychological factors bearing a differential risk for and contributing to the maintenance of substance addictions. One promising line of research focuses on empathic abilities. Empathy is an important domain of social cognition, enabling the person to infer, understand, and sympathize with the emotions of another person (88, 89). It is a multidimensional concept including the two facets cognitive empathy – the ability to infer the emotional state of another person – and emotional empathy, which describes affective reactions to the
emotions of another person (90). Emotional empathy consists of the two subfacets empathic concern and personal distress. Individuals with high empathic concern tend to respond with feelings of compassion or sympathy when another person experiences negative emotions. In contrast, individuals with high personal distress are prone to react with high arousal, aversive self-focused emotions, and social withdrawal (91).

Analogous to substance use disorders, sex differences in empathy have been observed with females typically displaying higher levels of empathic abilities (92, 93). For example, Lee and colleagues examined adolescents via a forced-choice emotion discrimination task. Adolescent girls displayed faster and more sensitive perception of facial emotions compared to adolescent boys (94).

Evidence from clinical studies indicates that substance use disorders are associated with impairments regarding empathic abilities. Most studies focus on cognitive empathy, often assessed via a behavioral paradigm investigating the ability to recognize facial expressions. For example, abstinent polysubstance users displayed poorer recognition of facial expressions of anger, disgust, fear, and sadness in comparison to controls. Measures of quantity and duration of substance use predicted poorer recognition of specific emotional facial expressions (95). Cocaine-addicted individuals showed significant impairments in the recognition of fear and anger in comparison to healthy controls (96). Townshend and Duka found impairments in the recognition of emotional facial expressions in alcohol-addicted patients compared to controls. Most notably, they reported an enhanced fear recognition in patients which was associated with the number of previous detoxifications (97).

Kornreich and colleagues reported that regarding the decoding of emotional facial expressions, accuracy scores were significantly lower in recently detoxified alcohol-addicted patients and subjects with both alcohol and opiate addiction antecedents compared to methadone-maintained opiate-addicted patients and detoxified opiate-addicted patients, which in turn had significantly lower scores than controls (98). These results signify that both alcohol addiction and opiate addiction are associated with impairments in cognitive empathy, the former to a greater extent than the latter. Consistently, McDonald and colleagues found impaired emotion perception in methadone- or buprenorphine-maintained opiate-addicted patients compared to controls and abstinent patients (99).

In addition to studies regarding deficits in cognitive empathy, previous research indicates that substance addictions are associated with impairments in emotional empathy as well.
For example, Preller and colleagues reported that cocaine-addicted patients showed less emotional empathy compared to controls (100). Alcohol-addicted patients were also found to exhibit impairments in emotional empathy (101). Ferrari and colleagues investigated empathy in a group of polysubstance users that included opiate-addicted patients and reported that substance-using subjects displayed lower scores in the factor emotional empathy of the empathy quotient compared to controls (102). Tomei and colleagues (103) found a specific profile of deficits in emotional empathy in methadone-maintained opiate-addicted patients with lower empathic concern and higher personal distress compared to controls.

Studies focusing on personality traits linked with the etiology and symptomatology of substance use disorders (104) may add to our understanding of the association between substance use disorders and empathy impairments. Personality traits are biologically based stable dispositions (105) and represent individual differences in cognitive thinking patterns, behavioral, and emotional tendencies. Empathic abilities and personality traits are related constructs. The personality dimension agreeableness is linked with cognitive empathy as well as empathic concern while neuroticism is associated with personal distress (106). In a meta-analysis conducted by Malouff and colleagues, an association of problematic alcohol consumption with a personality profile of low conscientiousness, low agreeableness and high neuroticism was reported (107). This corresponds to the studies outlined above pointing to low cognitive empathy, low empathic concern and high personal distress in patients with substance use disorders. In early adulthood from ages 18 to 35, changes in neuroticism and impulsivity were found to be associated with changes in problematic alcohol involvement, such that individuals with sharper declines in these personality traits were more likely to display steeper decreases regarding problematic alcohol consumption (108). Personality traits may also inform on motives for substance use (109). In a study examining young adults, aged 18 to 25 years old, individuals with high neuroticism were likely to engage in risky behaviors such as alcohol use in order to cope with aversive mood states (110). Coping motives, in turn, are associated with higher substance use (111).

1.6 Association of testosterone with empathic abilities

It is crucial to investigate possible associations and interactions of biological and psychological factors relevant for the etiology, maintenance, and recovery of substance
addiction in order to fully understand these severe disorders and to be able to treat them adequately. Regarding empathy impairments in substance addiction, previous research suggests the assumption that testosterone may be a possible physiological correlate and biomarker. Testosterone is linked with aggression and dominance behavior (30, 112). Studies investigating the role of prenatal hormone exposure found evidence for a negative impact of high fetal testosterone on social cognition. The hormone seems to have an effect on an organizational level by influencing fetal brain development (113, 114). A field study of Knickmeyer and colleagues showed a negative association between fetal testosterone in male and female children and the quality of social relationships at the age of four, obtained via self-report questionnaire completed by the children’s mothers (115). Chapman and colleagues found significant negative correlations between fetal testosterone and theory of mind abilities in six to eight years old children as well as fetal testosterone and empathy measured via a questionnaire completed by the children’s mothers (116).

Furthermore, testosterone has specific activational effects (117) which impair current performance regarding social cognition. Testosterone administration studies found evidence for impairments in emotional as well as cognitive empathy after the application of the hormone. Hermans and colleagues reported a decrease in facial mimicry after testosterone administration. Facial mimicry can be interpreted as a component of emotional empathy (118). Van Honk and colleagues found that testosterone application impaired cognitive empathy assessed via the Reading the Mind in the Eyes test, a behavioral task in which emotions have to be inferred from the eye-region of a face. This effect was predicted by second to fourth digit (2D:4D) ratio, the ratio between the length of the index and ring finger, a proxy of fetal testosterone. The group argued that early neurodevelopmental effects of testosterone might facilitate the activational effects of this hormone in adulthood (119).

Depending on prenatal sex hormone priming, testosterone application was reported to moderate the effect of the social environment on trust in women (120). In this study using the economic trust game, one-shot games modeling trust problems in relations between strangers were compared with repeated games modeling trust problems in ongoing relations between partners. Consistent with theoretical assumptions, subjects were more trustful in the latter condition. However, after testosterone administration, this effect disappeared in women prenatally highly primed by testosterone.
Bos and colleagues examined young women during the Reading the Mind in the Eyes test using functional magnetic resonance imaging. They reported that testosterone administration altered functional connectivity of the left inferior frontal gyrus with the anterior cingulate cortex and the supplementary motor area during the emotion recognition test performance, independent of 2D:4D ratio. This network is believed to underlie the integration and selection of sensory information and to play a crucial role regarding cognitive empathic behavior (121). Van Honk and Schutter found that the administration of testosterone reduced the detection of socially corrective signals. In their study, the recognition of facial expressions of anger was impaired in female subjects after the application of testosterone. The authors conclude that this effect may predispose individuals to antisocial behavior (122).

Summing up, previous research supports the assumption that testosterone plays an important role in regard to empathy impairments, both with respect to current performance and long-term deficits. However, this association has not been investigated yet in regard to specific empathy impairments in substance-addicted patients.

1.7 Aims of the two studies

In the two studies presented, testosterone levels in addicted patients are investigated during withdrawal and compared to the levels of healthy control subjects. Furthermore, possible associations between testosterone and other biological as well as psychological factors are examined. Study 1 focuses on testosterone and associated factors in alcohol addiction. Testosterone levels in alcohol-addicted patients during withdrawal are compared to the levels of healthy controls. Possible alterations of testosterone levels during alcohol withdrawal are examined. Furthermore, associations between testosterone, BDNF, and the symptomatology of alcohol withdrawal are investigated, as well as associations between these factors in subgroups of patients with high versus low HPA activity.

Study 2 focuses on testosterone as a possible biomarker of empathy impairments in opiate addiction. Testosterone levels in opiate-addicted patients during early withdrawal are compared to the levels of healthy controls. Furthermore, empathic abilities in opiate-addicted patients are compared to those of controls to examine whether patients show impairments in cognitive and emotional empathy. It is hypothesized that opiate-addicted patients display lower cognitive empathy and a specific pattern of deviations in emotional
empathy with lower empathic concern and higher personal distress compared to controls. Moreover, possible associations between testosterone levels and empathic abilities among the patients’ group are explored.
2. Study 1 – Association of testosterone and BDNF serum levels with craving during alcohol withdrawal

2.1 Methods

2.1.1 Sample

The sample contained 99 male patients (age mean 42.22 years, SD 7.83) who fulfilled the diagnostic criteria of alcohol addiction according to the International Classification of Diseases, 10th Edition (ICD-10) and the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) and 17 healthy male control subjects (age mean 44.41 years, SD 9.63). All patients were admitted for detoxification treatment (Klinik für Psychiatrie, Psychotherapie und Psychosomatik, Bezirksklinikum Obermain, Kutzenberg). Exclusion criteria were comorbid psychiatric disorder, substance abuse or addiction apart from alcohol and nicotine, severe somatic illness (in particular any type of cancer), autoimmune disease, HPA axis deregulation, and previous cerebral damage (e.g. ischemia or cerebral hemorrhage). Patients received carbamazepine and/or clomethiazole for the treatment of alcohol withdrawal symptomatology. The patients’ group consisted of smokers and non-smokers (10 non-smokers, 81 smokers, 6 former smokers, 2 unknown). Controls were screened for alcohol abuse and addiction using the CAGE questionnaire (123) and the Alcohol Use Disorder Identification Test – Alcohol Consumption Questions (AUDIT-C) (124). A score of 0 points in the CAGE questionnaire and a score below 3 points in the AUDIT-C were required for inclusion in the control group. Controls were negative for alcohol abuse, alcohol addiction, and any other Axis-I psychiatric disorder according to ICD-10 or DSM-IV. Controls received no psychopharmacological treatment and did not suffer from any current somatic disease. In the control group, there were 3 smokers and 14 former smokers. The study was part of a large research project (Studies in Neuroendocrinology and Neurogenetics in Alcoholism, NENA) (125) which was approved by the ethics committee of the Friedrich-Alexander-University of Erlangen-Nuremberg. All participants gave written informed consent.

2.1.2 Measures and procedure

The blood samples were obtained between 8:00 and 10:00 a.m. In the patients’ group, blood samples were taken before the patients received their morning medication on day 1, day 7,
and day 14 of alcohol withdrawal. BDNF, testosterone, and cortisol serum levels were assessed using enzyme-linked immunosorbent assay (ELISA). All patients underwent a physical examination, routine laboratory testing, and urine drug screening. Breath alcohol concentration was measured on admission and during alcohol withdrawal. Additional data such as age, body mass index (BMI), years of drinking, and daily intake of alcohol in grams were obtained by interview. Affective symptoms were assessed via Beck’s Depression Inventory (BDI) (126) and the State and Trait Anxiety Inventory (STAI-I and STAI-II) (127) immediately after taking the blood samples. Intensity of alcohol craving was measured via the Obsessive Compulsive Drinking Scale (OCDS) (128). Severity of alcohol withdrawal was assessed via the Clinical Institute Withdrawal Assessment for Alcohol (CIWA-Ar) (129). The severity of alcohol addiction was measured via the Severity of Alcohol Dependence (SESA) questionnaire and its subscales (130) which assess narrowing of the drinking repertoire (SESA-XE), somatic withdrawal symptoms (SESA-XK), alcohol consumption to avoid withdrawal symptoms (SESA-XV), psychological withdrawal symptoms (SESA-XP), increase of tolerance (SESA-XT), extreme increase of tolerance (SESA-XTE), and decrease of tolerance (SESA-XTU).

2.1.3 Data analysis

Because the testosterone levels were not normally distributed according to the Kolmogorov-Smirnov test, non-parametric statistics were applied. In order to assess alterations of testosterone serum levels during alcohol withdrawal three difference values were calculated, mirroring the alterations of testosterone serum levels from day 1 to day 7, from day 7 to day 14, and from day 1 to day 14. In order to assess the possible impact of HPA activity a median split was calculated to diverge patients with high (subgroup A) and low (subgroup B) cortisol serum levels during alcohol withdrawal. Spearman’s correlation coefficient was used to compute correlations. The Friedman test was used to calculate mean differences between the testosterone serum levels of the alcohol-addicted patients on day 1, 7, and 14. Group differences between the control group and the patients’ group were calculated by the Mann-Whitney-U-test. Significance level was set to 0.05. All calculations were computed with SPSS 20.
Table 1: Sample characteristics of the alcohol-addicted patients and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Patients’ group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>42.22 ± 7.83</td>
<td>44.41 ± 9.63</td>
<td>n.s.</td>
</tr>
<tr>
<td>BMI</td>
<td>24.62 ± 3.60</td>
<td>26.44 ± 4.58</td>
<td>n.s.</td>
</tr>
<tr>
<td>Years of drinking</td>
<td>9.42 ± 7.67</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Daily intake in grams</td>
<td>194.90 ± 83.42</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>OCDS total score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>19.80 ± 6.70</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Day 7</td>
<td>10.92 ± 7.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>9.88 ± 6.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PACS score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>16.15 ± 7.56</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Day 7</td>
<td>6.27 ± 5.78</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>5.07 ± 6.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CIWA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>15.64 ± 4.23</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Day 7</td>
<td>12.84 ± 2.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>12.33 ± 2.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SESA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>48.92 ± 18.05</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>BDI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>17.40 ± 8.75</td>
<td>3.00 ± 3.77</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Day 7</td>
<td>8.23 ± 7.60</td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td>Day 14</td>
<td>5.97 ± 7.26</td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>STAI-I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>48.78 ± 12.59</td>
<td>31.47 ± 5.34</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Day 7</td>
<td>37.64 ± 10.96</td>
<td></td>
<td>0.043</td>
</tr>
<tr>
<td>Day 14</td>
<td>37.70 ± 11.96</td>
<td></td>
<td>0.006</td>
</tr>
<tr>
<td>STAI-II</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1</td>
<td>47.86 ± 10.83</td>
<td>30.65 ± 6.94</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

2.2 Results

2.2.1 Group differences regarding testosterone between the patients’ group and the control group

In the patients’ group, there were higher testosterone levels compared to the control group (day 1: \( U = -3.652, p < 0.001 \), day 7: \( U = 3.238, p = 0.001 \), day 14: \( U = 3.019, p = 0.003 \)).

2.2.2 Group differences regarding testosterone between subgroup A and subgroup B

In subgroup A (high HPA axis activity), there were higher testosterone levels compared to subgroup B (low HPA axis activity) on day 1 (\( U = -2.58, p = 0.010 \)).
Table 2: Group comparisons regarding testosterone, BDNF, and cortisol serum levels between the group of alcohol-addicted patients and the healthy control group

<table>
<thead>
<tr>
<th></th>
<th>Patients’ group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Testosterone (ng/ml)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1:</td>
<td>10.05 ± 11.42</td>
<td>5.11 ± 5.42</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Day 7:</td>
<td>6.06 ± 12.69</td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>Day 14:</td>
<td>5.64 ± 8.69</td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td><strong>BDNF (pg/ml)</strong></td>
<td></td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>Day 1:</td>
<td>641.67 ± 511.71</td>
<td>728.84 ± 383.38</td>
<td></td>
</tr>
<tr>
<td>Day 7:</td>
<td>556.33 ± 400.92</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 14:</td>
<td>651.74 ± 519.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cortisol (nmol/l)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 1:</td>
<td>281.85 ± 133.98</td>
<td>183.00 ± 87.66</td>
<td>0.002</td>
</tr>
<tr>
<td>Day 7:</td>
<td>204.16 ± 101.22</td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>Day 14:</td>
<td>261.82 ± 182.02</td>
<td></td>
<td>0.003</td>
</tr>
</tbody>
</table>

2.2.3 Alterations of testosterone levels during alcohol withdrawal in the patients’ group

There was a decrease of testosterone serum levels in the patients’ group during alcohol withdrawal ($\chi^2 = 32.23$, $p < 0.001$).

Figure 1: Testosterone serum levels during alcohol withdrawal (day 1, day 7, day 14) in the group of alcohol-addicted patients compared to the testosterone levels of the healthy control group (C)

2.2.4 Association of testosterone with BDNF and cortisol among the patients’ group

Among the patients’ group, there was a negative correlation of the decrease of testosterone serum levels from day 1 to day 7 of alcohol withdrawal and BDNF serum levels on day 1 ($\rho = -0.268$, $p = 0.008$). Moreover, among the patients’ group, testosterone serum levels
correlated with cortisol serum levels on day 1 (rho = 0.243, p = 0.017) and day 14 (rho = 0.366, p < 0.001) of alcohol withdrawal.

2.2.5 Association of testosterone with severity of alcohol addiction
Among the patients’ group, the decrease of testosterone serum levels from day 1 to day 14 was associated with the scores on the XTU subscale of the SESA (rho = 0.227, p = 0.026).

2.2.6 Association of testosterone with alcohol craving
Among the patients’ group, the decrease of testosterone serum levels from day 1 to day 7 was positively associated with alcohol craving measured via the OCDS on day 7 (rho = 0.248, p = 0.014) and with scores in the compulsive subscale of the OCDS on day 7 (rho = 0.324, p = 0.001).

2.2.7 Associations of testosterone, BDNF, and craving in subgroup A
In subgroup A (high HPA axis activity), the decrease of testosterone serum levels from day 1 to day 7 and from day 7 to day 14 correlated negatively with BDNF serum levels (rho = -0.369, p = 0.013) and positively with compulsive alcohol craving (rho = 0.417, p = 0.004) on day 7 of alcohol withdrawal. In subgroup A, the BDNF serum levels correlated positively with the OCDS total score (rho = 0.429, p = 0.003), scores in the obsessive subscale of the OCDS (rho = 0.352, p = 0.018), and scores in the compulsive subscale (rho = 0.383, p = 0.009) on day 1.
3. Study 2 – Positive association of personal distress with testosterone in opiate-addicted patients

3.1 Methods

3.1.1 Sample

The sample consisted of 27 patients (21 males; age mean 41.67 years, SD 8.814) who fulfilled the diagnostic criteria of opiate addiction according to ICD-10 and DSM-IV and 31 healthy control subjects (23 males; age mean 40.77 years, SD 8.401). All patients participated in the structured diacetylmorphine maintenance program of the Hanover Medical School receiving injectable diacetylmorphine once or twice per day. Opiate addiction was the principal diagnosis for all patients in the sample. There was no major psychiatric comorbidity (i.e. no patients with acute psychosis, acute manic episode or severe depression). Exclusion criteria were human immunodeficiency virus (HIV) infections, intoxication, and positive breath alcohol concentrations. The control subjects were recruited from the community. They were matched in age, sex, and educational level to the opiate-addicted patients to address potential sources of bias. All controls were examined regarding psychiatric disorders using the Structured Clinical Interview for DSM-IV (131) and were negative for all Axis-I psychiatric disorders. They were not treated with psychopharmacological medication or psychotherapy and did not suffer from any current somatic disease. The study was approved by the ethics committee of the Hanover Medical School. All participants gave written informed consent.

3.1.2 Measures and procedure

Testosterone was measured in saliva, a robust and reliable method (132, 133) that is especially suitable for the investigated group of opiate-addicted patients due to the noninvasive and stress-free collection of samples. As the stimulation of saliva flow (132) and the use of sampling devices with cotton-based materials absorbing the saliva (134) can interfere with the results, polypropylene SaliCaps® and straws were used. Because of circadian variation of testosterone levels (135), the sampling was conducted at the same time of day for all subjects. Complying with the instructions of the manufacturer (IBL international), five salivary samples were obtained and pooled before measuring testosterone levels. This procedure was implemented to address the potential bias of rapid fluctuations of salivary testosterone concentrations (136); the mixture of the five samples
represents the hormone activity over the period of sampling. The samples were taken between 8:30 and 10:30 a.m. with a time frame of 20 minutes after each sample. In the patients’ group, the first sample was obtained directly before the patients received their regular dose of diacetylmorphine. As the effect of opiates on testosterone levels usually becomes apparent several hours after administration with a maximum effect after four to six hours (137), the salivary samples were obtained before the immediate effect of diacetylmorphine had set in and testosterone levels during early opiate withdrawal were depicted. The samples were stored frozen at -80° prior to analysis. Testosterone levels were measured using enzyme-linked immunosorbent assay (ELISA).

Demographic data were obtained by interview and self-report after taking the second salivary sample. Subsequently, empathy was measured via the German version of the Interpersonal Reactivity Index (138), the Saarbrücker Persönlichkeitsfragebogen (SPF) (139). The SPF is a self-report questionnaire assessing cognitive empathy (perspective taking) and emotional empathy (empathic concern, personal distress and fantasy). The perspective taking scale measures the ability to infer the perspective of another person. The empathic concern scale assesses the tendency to feel sympathy and compassion toward another person who experiences negative emotions, while the personal distress scale measures the tendency to react with self-focused negative emotional states. The fantasy scale assesses the ability to identify with fictional characters.

3.1.3 Data analysis

An exploratory factor analysis (maximum likelihood extraction method, varimax rotation) combined with Cattell’s scree plot test and the Kaiser-Meyer-Olkin test was performed on the whole sample to verify conformity with the original dimensions of the Interpersonal Reactivity Index (138). Reliabilities were assessed via Cronbach’s alpha. The t-test for independent samples and Pearson’s chi-squared test were conducted to examine the matching of the control group to the patients’ group. The t-test for independent samples was used to calculate group differences between the patients’ group and the control group (one-tailed for empathy, two-tailed for testosterone). Significance level was set to 0.05. The homogeneity of the variances was tested via the Levene-test. The Welch-t-test was used to calculate the group differences regarding testosterone, because the variances were not homogenous for this variable. Cohen’s d was calculated to establish effect sizes. Pearson’s
correlation coefficient was used to compute correlations (all two-tailed). In addition, partial correlations were conducted. All calculations were computed with SPSS 24.

Table 3: Sample characteristics of the opiate-addicted patients and healthy controls

<table>
<thead>
<tr>
<th></th>
<th>Patients’ group</th>
<th>Control group</th>
<th>t/χ²</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age in years</strong></td>
<td>41.67 (±8.814)</td>
<td>40.77 (±8.401)</td>
<td>0.394</td>
<td>56</td>
<td>n.s.</td>
</tr>
<tr>
<td><strong>Sex:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21 (77.8%)</td>
<td>23 (74.2%)</td>
<td>0.101</td>
<td>1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Female</td>
<td>6 (22.2%)</td>
<td>8 (25.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Educational level:</strong></td>
<td></td>
<td></td>
<td>-1.662</td>
<td>56</td>
<td>n.s.</td>
</tr>
<tr>
<td>Main school/no degree</td>
<td>3 (11.1%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main school with degree</td>
<td>9 (33.3%)</td>
<td>7 (22.6%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle school/no degree</td>
<td>0 (0%)</td>
<td>2 (6.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle school with degree</td>
<td>10 (37%)</td>
<td>14 (45.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Academic high school/no degree</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Academic high school with degree</td>
<td>4 (14.8%)</td>
<td>8 (25.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other substance use disorders:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol addiction (F10.2)</td>
<td>4 (14.8%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol abuse (F10.1)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannabinoid addiction (F12.2)</td>
<td>8 (29.6%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannabinoid abuse (F12.1)</td>
<td>7 (25.9%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzodiazepine addiction (F13.2)</td>
<td>14 (51.9%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzodiazepine abuse (F13.1)</td>
<td>3 (11.1%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine addiction (F14.2)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine abuse (F14.1)</td>
<td>4 (14.8%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Other psychiatric disorders:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic mental disorder (F06.9)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizophrenia (F20.0)</td>
<td>2 (7.4%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delusional disorder (F22)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bipolar disorder (F31)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent depressive disorder (F33)</td>
<td>4 (14.8%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed anxiety and depressive disorder (F41.2)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTSD (F43.1)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD (F90)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined personality disorder (F61)</td>
<td>12 (44.4%)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borderline pers. disorder (F60.31)</td>
<td>6 (22.2%)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Smoking status:</strong></td>
<td></td>
<td></td>
<td>24.035</td>
<td>1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Active smoker</td>
<td>25 (92.6%)</td>
<td>9 (29%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>2 (7.4%)</td>
<td>22 (71%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chronic hepatitis C virus infection:</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>21 (77.8%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-infected</td>
<td>6 (22.2%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2 Results

3.2.1 Preliminary analyses

As the fantasy scale of the SPF was not included in the hypotheses, only the items belonging to the remaining scales (perspective taking, empathic concern, and personal distress) were included in the factor analysis. The Kaiser-Meyer-Olkin test confirmed adequacy of the sample (KMO = 0.706). Cattell’s scree plot pointed toward three factors. 62.33% of total variance was explained. The original dimensions were confirmed (perspective taking: items 4, 10, 14, 16; empathic concern: items 1, 5, 11; personal distress: items 3, 6, 8, 13). One item (9) of the empathic concern subscale had to be eliminated because it was loading too high on factor one (perspective taking) and because of its correspondence to the perspective taking scale as well as the fantasy scale in terms of content. The reliabilities of the dimensions generated by the factor analysis were then tested via Cronbach’s alpha tests. The reliability of the perspective taking scale was good (α = 0.805). The empathic concern subscale (α = 0.698) and the personal distress subscale (α = 0.675) had acceptable reliabilities. All items had acceptable to high item-scale-correlations. Subsequently, aggregated scores for each scale were computed.

3.2.2 Group differences regarding testosterone between the patients’ group and the control group

In the patients’ group, there were higher testosterone levels compared to the control group with a large effect size (t = 3.955, p < 0.001, df = 34.006, d = 1.093).

Table 4: Group comparisons regarding testosterone levels and empathy scores between the group of opiate-addicted patients and the healthy control group

<table>
<thead>
<tr>
<th></th>
<th>Patients’ group</th>
<th>Control group</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (pg/ml)</td>
<td>768.78 ± 497.427</td>
<td>361.81 ± 210.231</td>
<td>3.955</td>
<td>34.006</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Personal Distress</td>
<td>11.41 ± 3.165</td>
<td>8.84 ± 3.132</td>
<td>3.100</td>
<td>56</td>
<td>0.002</td>
</tr>
<tr>
<td>Empathic concern</td>
<td>10.89 ± 2.667</td>
<td>11.52 ± 2.204</td>
<td>-0.981</td>
<td>56</td>
<td>n.s.</td>
</tr>
<tr>
<td>Perspective Taking</td>
<td>14.41 ± 3.092</td>
<td>15.65 ± 2.916</td>
<td>-1.568</td>
<td>56</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
3.2.3 Group differences regarding empathy between the patients’ group and the control group

In the patients’ group, there were higher scores in the personal distress scale of the SPF compared to the control group with a large effect size ($t = 3.100, p = 0.002, df = 56, d = 0.817$).

3.2.4 Association of testosterone with empathy among the patients’ group

There was a positive correlation of testosterone and personal distress among the patients’ group ($r = 0.399, p = 0.039, n = 27$). Hepatitis ($r = 0.532, p < 0.001, n = 58$) and smoking status ($r = 0.325, p = 0.013, n = 58$) correlated with testosterone in the whole sample. Hepatitis correlated with personal distress in the whole sample as well ($r = 0.303, p = 0.021, n = 58$). Therefore, a partial correlation of testosterone and personal distress with hepatitis and smoking status as control variables was conducted among the patients’ group. The positive correlation between testosterone and personal distress remained significant ($r = 0.434, p = 0.030, n = 27, df = 23$).

3.2.5 Complementary results

As the number of female participants differed in both groups and because there are natural sex differences regarding testosterone levels (13, 14), group analyses regarding male patients ($n = 21$) and controls ($n = 23$) only were conducted. There were analogous group differences regarding personal distress ($t = 3.752, p < 0.001, df = 42$) as well as testosterone ($t = 4.068, p < 0.001, df = 26.073$). In the subgroup of male patients, a comparable correlation of testosterone and personal distress ($r = 0.545, p = 0.011, n = 21$) was found. The correlation between the two variables remained significant when conducting a partial correlation with hepatitis and smoking status as control variables ($r = 0.570, p = 0.011, n = 21, df = 17$).
4. Discussion

In the two studies presented, testosterone levels in addicted patients during withdrawal were compared to the levels of healthy control subjects. Furthermore, associations between testosterone and other biological as well as psychological factors were examined.

In study 1, higher testosterone serum levels in alcohol-addicted patients during alcohol withdrawal were found compared to healthy controls. The testosterone levels of the alcohol-addicted patients decreased significantly during withdrawal. The decrease of testosterone levels in the patients’ group during early withdrawal was negatively associated with the BDNF levels at the beginning of withdrawal and positively associated with alcohol craving in the middle of the examined withdrawal period. The decrease of testosterone levels throughout the observed period of alcohol withdrawal was positively associated with the scores on a subscale of the addiction severity questionnaire. In the subgroup of patients with higher cortisol serum levels, mirroring high activity of the HPA axis, the decrease of testosterone levels throughout alcohol withdrawal was negatively associated with BDNF levels and positively associated with craving in the middle of the examined withdrawal period. Moreover, in this subgroup the BDNF serum levels were positively associated with alcohol craving at the beginning of withdrawal.

In study 2, higher salivary testosterone levels in opiate-addicted patients during early withdrawal were found compared to healthy controls. The examined opiate-addicted patients displayed impairments in empathic abilities, namely higher personal distress, compared to controls. Moreover, a positive association between testosterone and personal distress among the patients’ group was found.

4.1 Testosterone during alcohol and opiate withdrawal

Higher testosterone levels in addicted patients in comparison to the testosterone levels of controls were found in both studies. Regarding alcohol addiction, this corresponds to previous preclinical research reporting higher testosterone levels in alcohol-preferring compared to non-preferring rats (15) and a positive association between testosterone release and alcohol consumption in alcohol-preferring rats (16). Contrastingly, clinical studies on chronic alcohol consumption typically reported lower levels in patients compared to controls (22, 23). These seemingly contradicting findings can be explained by the time
frame of study 1, as the testosterone levels of the alcohol-addicted patients were obtained during withdrawal. The results are consistent with findings of previous clinical studies reporting higher testosterone levels in alcohol-addicted patients during and after withdrawal compared to controls (24, 25). In the study conducted by Walter and colleagues, the plasma testosterone levels of alcohol-addicted patients were higher compared to controls after six weeks of abstinence; higher levels before and after withdrawal in the patients’ group were associated with higher alcohol consumption before detoxification (24). Hasselblatt and colleagues investigated alcohol-addicted patients over a period of 18 weeks of controlled abstinence. The serum testosterone levels of the patients’ group were higher compared to controls on the second day of abstinence and stayed elevated over the whole observation period. The authors conclude that these findings point to a profound disturbance of the HPG axis in alcohol addiction that persists after cessation of alcohol consumption (25). Thus, the high testosterone levels during alcohol withdrawal in the patients’ group found in study 1 may indicate a dysregulation of the HPG axis in alcohol-addicted patients as described by Rachdaoui and Sarkar (140).

Regarding opiate addiction, previous clinical studies on chronic use also found lower levels in patients compared to controls (26, 27). But analogous to study 1, the testosterone levels of the patients’ group in study 2 were obtained during the withdrawal period, in this case during early opiate withdrawal. Thus, the results may as well reflect a dysregulation of the HPG axis in addicted patients.

Brown and colleagues reported that HPA axis dynamics change drastically in opiate withdrawal. Opiate administration results in a subsequent suppression of HPA axis activity; but during opiate withdrawal, the HPA axis becomes overstimulated, resulting in hypercortisolism and possibly reinforcing relapse behavior (137). With respect to the findings of study 2, it could be argued that similar effects occur regarding the HPG axis: Acute administration of opiates leads to a subsequent suppression of testosterone several hours later, while opiate withdrawal causes a shift in HPG axis dynamics resulting in an overstimulation and higher testosterone levels. This hypothesis is consistent with a recent study of Janke and colleagues (unpublished results) reporting higher testosterone levels in opiate-addicted patients before the administration of their regular doses of diacetylmorphine compared to the testosterone levels several hours later. Moreover, in this study a significant positive correlation of testosterone levels before the administration of
diacetylmorphine with opiate craving assessed via self-report was found. This finding corresponds to preclinical research reporting a link between testosterone and withdrawal symptomatology in rats during opiate withdrawal (28).

The results of study 1 indicate that the testosterone levels of alcohol-addicted patients are especially high during early withdrawal and decrease subsequently. A positive association between the decrease of testosterone levels and alcohol craving measured via the OCDS was found among the patients’ group. It has been reported repeatedly that OCDS scores can predict addiction treatment outcome (141, 142, 143, 144). Moreover, the OCDS is positively associated with different well-established addiction severity measures and was itself suggested to be utilized as an instrument to assess alcohol addiction severity (145, 146, 147). For instance, the OCDS total score was found to correlate with the alcohol subscale of the Addiction Severity Index and the Alcohol Dependence Scale in a sample of alcohol-addicted patients (146). Therefore, it could be argued that HPG axis dysregulation, as indicated by high testosterone levels during early withdrawal followed by a steep decrease, is linked with addiction severity. Consistently, in study 1 the decrease of testosterone levels throughout alcohol withdrawal was not only positively associated with OCDS scores but also with the scores on the XTU subscale of the SESA questionnaire assessing alcohol addiction severity.

Because testosterone is associated with impulsivity (29, 30), this assumption corresponds to the results of previous studies linking impulsivity with severity of substance use disorders (148, 149). For example, in alcohol-addicted patients, self-reported impulsivity and cognitive impulsivity assessed by a behavioral measure were positively associated with emotional craving in a study conducted by Joos and colleagues. These associations seemed to be more pronounced in severely addicted patients (148). In a study by Papachristou and colleagues, impulsivity scores of heavy drinkers were higher compared to the scores of light drinkers. Heavy drinkers with ineffective response inhibition – which is one aspect of impulsivity – displayed more craving after exposure to alcohol cues compared to heavy drinkers with adequate response inhibition (149). Furthermore, impulsivity was linked to unfavorable addiction treatment outcomes such as difficulties in achieving and maintaining abstinence (150). Against this background, it could be argued that high testosterone levels during early withdrawal, reflecting HPG axis dysregulation, might be considered a marker for severe addiction.
Summing up, the results of studies 1 and 2 support the assumption that the sex hormone testosterone plays an important role in regard to substance addiction. While being preliminary, the results indicate a significant involvement of testosterone in the withdrawal period of alcohol and opiate addiction.

When taking into account previous studies, the following hypotheses for further research can be derived from the results of studies 1 and 2: After the development of a substance addiction the HPG axis may become increasingly dysregulated, as indicated by suppressed testosterone levels during chronic substance use and elevated testosterone levels during early withdrawal followed by a steep decrease. This dysregulation of the HPG axis might be more pronounced in more severely addicted patients. Thus, high testosterone levels during early withdrawal might be considered a marker for severe addiction.

4.2 Interplay of testosterone and BDNF in alcohol addiction

There were no significant group differences between the patients’ group and the control group regarding BDNF serum levels in study 1. Contrastingly, previous studies typically reported lower BDNF levels in alcohol-addicted patients compared to controls (53, 54) suggesting that chronic alcohol consumption leads to a reduction in BDNF levels. However, study 1 investigated BDNF levels throughout the course of alcohol withdrawal which may in part explain these diverging results: Other studies found increased BDNF levels after alcohol detoxification compared to baseline levels (55), and the BDNF levels of alcohol-addicted patients after withdrawal were reported to be comparable to the levels of controls (54).

The results of study 1 support the assumption of a relevant interplay of BDNF and testosterone in regard to alcohol addiction. The decrease of testosterone levels in the group of alcohol-addicted patients during early withdrawal was negatively associated with the BDNF levels at the beginning of alcohol withdrawal and positively associated with alcohol craving in the middle of the examined withdrawal period.

Similar results were found regarding the subgroup of patients with a high activity of the HPA axis, also pointing to an interaction of testosterone and BDNF that is functionally relevant with respect to alcohol withdrawal symptomatology: The decrease of testosterone levels throughout the withdrawal period was negatively associated with BDNF levels and positively associated with craving in the middle of the observed withdrawal period. However, in this subgroup the BDNF serum levels were positively associated with alcohol craving at the
beginning of withdrawal. Thus, alcohol craving during alcohol withdrawal seems to be influenced by a complex interplay of testosterone, BDNF, and HPA axis activity. Interactions between BDNF and testosterone (65, 66, 67, 68) as well as interactions between BDNF and the HPA axis (82, 83, 84, 85, 87) have been reported repeatedly, supporting this assumption. Furthermore, the findings of study 1 indicate that there may be a relevant association between HPG axis dysregulation and HPA axis activity in alcohol addiction: Cortisol was positively associated with testosterone in the patients’ group at the beginning and end of withdrawal. Cortisol levels were higher in the patients’ group compared to controls at the beginning and end of withdrawal, similar to group differences regarding testosterone levels. The testosterone levels of the subgroup with high HPA axis activity were higher compared to the subgroup with low HPA axis activity at the beginning of withdrawal. Thus, patients with severe alcohol addiction might be characterized by HPG axis dysregulation as well as high HPA axis activity during withdrawal. The results are consistent with studies investigating the interplay of the HPG axis and the HPA axis (74, 75, 76) and correspond to research on functional interactions of the HPG and the HPA axis with respect to alcohol craving (80) and voluntary alcohol consumption in rats (81).

Summing up, the interplay of testosterone and BDNF seems to play an important part with respect to alcohol craving during alcohol withdrawal. While being preliminary, the results indicate that HPA axis activity is associated with HPG axis dysregulation and involved in the interplay of testosterone and BDNF in regard to alcohol withdrawal symptomatology.

4.3 Cognitive and emotional empathy in opiate addiction

In contrast to two previous studies (98, 99), the investigated opiate-addicted patients in study 2 did not display impairments in cognitive empathy compared to controls. One possible explanation for the differing findings may be the assessment of cognitive empathy via self-report in our study, while the above-mentioned earlier studies used behavioral paradigms. Self-reported cognitive empathy and empathic abilities assessed with a behavioral paradigm typically correlate only poorly (151). Melchers and colleagues recommend using questionnaire measures such as the Interpersonal Reactivity Index in clinical research (151). Behavioral measures likely capture different endophenotypes of empathic abilities: In the two prior studies reporting impairments in cognitive empathy in opiate-addicted patients (98, 99), emotion perception was investigated with a behavioral
test, while study 2 assessed the self-concept of patients regarding their perceived ability to take the perspective of another person.

With respect to empathic concern, no significant group differences between the opiate-addicted patients and controls were found. This contrasts with the finding of lower empathic concern in methadone-maintained opiate-addicted patients compared to controls reported by Tomei and colleagues (103). The authors assessed empathic abilities via the Interpersonal Reactivity Index. The original version of the questionnaire (138) was used and translated to French. The SPF used in study 2 (139) is shorter than this version and contains different items. Moreover, Tomei and colleagues conducted a preliminary factor analysis which was followed by the reassignment of several items to and from the empathic concern subscale. Therefore, it is possible that the subscale used in their study was not congruent with the one used in study 2 which may be an explanation for the differing findings. Further research is needed to clarify the results.

The opiate-addicted patients in study 2 displayed higher levels of personal distress compared to controls. This is consistent with the finding of high personal distress in methadone-maintained patients reported by Tomei and colleagues (103). As personal distress is positively associated with neuroticism (106), the finding also corresponds to previous research linking high and problematic substance use with neuroticism (107, 108, 111).

Subjects with high personal distress have the tendency to exhibit maladaptive, self-focused affective responses to negative emotions in others which results in physiological over-arousal and behavioral withdrawal (91). Consistent with the finding of high personal distress in opiate-addicted patients in study 2, previous studies reported that opiate-addicted patients have an altered emotional experience, characterized by an increased response to unpleasant stimuli (152).

For the following reasons, personal distress may be relevant for the maintenance of the disorder. Opiate as well as alcohol use and personality traits associated with negative affect are closely linked (153, 154), possibly accounted for by a high sensitivity to the dampening effects of these substances (155, 156). Negative emotional states have been shown to trigger opiate craving in addicted patients, which can be interpreted in the context of self-medication (157). For instance, heroin craving was associated with increases in reports of feeling sad or angry in methadone-maintained cocaine- and heroin-abusing patients (158).
Similar findings were reported regarding alcohol-addicted patients (159). Opiate-addicted patients seem to lack adequate and adaptive coping skills (160). This possibly contributes to the tendency to use opiates as a dysfunctional strategy to cope with negative emotional states. The key mechanism in this respect is negative reinforcement (161, 162): Because of the experience that opiates are able to alleviate dysphoric or anxious moods, the individual closely associates opiates with the relief of negative emotional states. As a consequence, substance craving is likely to be triggered when aversive emotional states occur. Personal distress as a trait that is associated with habitually occurring negative emotions in social situations may therefore contribute to opiate craving and opiate-seeking behavior.

Using opiates as a dysfunctional coping strategy to alleviate negative emotional states seems to be a relevant factor concerning recovery from addiction. Hser reported that in comparison to recovered substance-addicted patients, non-recovered patients were significantly more likely to use substances to cope with stressful conditions (163). Therefore, recurring negative emotional states are considered important targets for the treatment of opiate-addicted patients to avoid relapse.

Summing up, the results of study 2 indicate that opiate-addicted patients may be characterized by high personal distress and as a consequence are prone to habitually experience high arousal and negative emotional states in social situations. Because opiate-addicted patients lack adequate coping skills, this is likely to recurrently evoke opiate craving. Personal distress may therefore be considered a relevant factor for the maintenance of opiate addiction and a fruitful target for treatment.

Opiate-addicted subjects with especially high personal distress possibly experience craving very frequently and strongly. Therefore, it can be argued that high personal distress might be considered a possible marker for severe opiate addiction. This hypothesis is consistent with the results of studies reporting a negative correlation between emotional intelligence and addiction severity (164, 165). Sanvicente-Vieira and colleagues recently drew a similar conclusion when examining social cognition in female cocaine-addicted patients: The authors stated that the sociocognitive impairments of the investigated patients were associated with craving and appeared to be related to addiction severity (166).
4.4 Positive association of testosterone with personal distress in opiate addiction

The observed association between personal distress and testosterone in opiate-addicted patients in study 2 points toward testosterone as a possible biomarker of empathy impairments in opiate-addicted patients, which has not been investigated before. The association between the two variables is consistent with previous research on possible negative long-term effects of fetal testosterone on social cognition (113, 115, 116) as well as testosterone administration studies with healthy subjects reporting impairments in emotional empathy after the application of the hormone (118).

Neuroimaging studies have shown that testosterone suppresses prefrontal restraining control on impulsive tendencies and enhances the activity of the amygdala (30). For example, testosterone was positively associated with amygdala activity as a response to fearful and angry facial expressions in healthy males (167). Impulsive behavior such as impulsive aggression is viewed as a consequence of impaired emotion regulation (168). Thus, testosterone seems to be important not only in regard to impulsivity but also in regard to emotion dysregulation. Personal distress is linked with low self-regulation (169) and malfunctioning emotion regulation (170) as well.

Against this background, the following study elucidating the possible role of excessive amygdala activity in the maintenance of opiate addiction is interesting: Schmidt and colleagues compared a group of opiate-addicted patients receiving heroin with opiate-addicted patients receiving saline and healthy control subjects in regard to the amygdala response to negative facial expressions (171). The opiate-addicted patients receiving saline displayed a significantly higher left amygdala activity as a response to fearful faces compared to healthy controls. Patients receiving heroin did not differ from controls in regard to their amygdala activity. Moreover, left amygdala activity was associated with state-anxiety among all patients and controls. It can be concluded that opiate-addicted patients seem to display an altered and excessive amygdala response to negative facial expressions that can be reduced to normal levels via opiate administration.

Further research could investigate whether this altered amygdala reactivity is linked with high personal distress: Subjects with high personal distress might habitually display excessive amygdala responses to perceived negative emotions of other persons. The ability of opiates to reduce excessive amygdala reactivity – which is possibly modulated by testosterone – and
to alleviate associated negative emotional states could play a critical role in the maintenance of opiate addiction via negative reinforcement.

4.5 Limitations

There are some limitations which may have impacted the results reported for study 1 and study 2. Both studies had an associative character allowing no causal conclusions to be drawn. Follow-up studies with larger sample sizes are warranted to corroborate the results. For study 1, a study setting measuring cortisol, BDNF, and testosterone levels at subsequent points during the day would have helped to investigate more clearly the interplay between testosterone, BDNF, and HPA axis activity.

For study 2, it would have been preferable to obtain all salivary samples before the patients received their regular doses of diacetylmorphine to ensure that indeed the testosterone levels during early withdrawal were measured. In the study design used, only the first salivary sample was obtained beforehand, which was due to difficulty of motivating these severely addicted patients to participate in the study before receiving diacetylmorphine. Because the effect of opiates on testosterone levels typically becomes apparent after several hours (137), testosterone levels during early withdrawal were reflected; nevertheless, the above-outlined design would have improved the clarity of the results and is recommended for possible follow-up studies. A study design assessing testosterone levels at subsequent points over the course of several days during opiate withdrawal and investigating possible associations with craving and withdrawal severity would have greatly increased the research value. In this case possible alterations of testosterone levels during the withdrawal period and the relevance of testosterone for withdrawal symptomatology – analogous to study 1 with respect to alcohol withdrawal – could have been investigated.

In study 1, only male subjects were investigated while in study 2 the number of included female patients was small. Therefore, the generalization of the results in regard to female addicted patients is difficult and further research is warranted in this respect.

In both studies, there were possible confounding variables which may have biased the results. In study 1, the investigated alcohol-addicted patients were treated with alcohol withdrawal medication. In study 2, several patients were treated with psychopharmacological medication, which was not monitored. In both studies, the groups
were heterogeneous regarding smoking status. These possible confounding factors should be taken into account when interpreting the results.

Moreover, in study 2 the sample contained patients suffering from comorbid psychiatric disorders, other substance addictions, and health problems. This is typical for severely addicted patients taking part in a structured diacetylmorphine-maintenance program (172). Hence, this can be considered an important strength of the study, increasing ecological validity. But on the downside, these comorbid disorders – even though opiate addiction was the principal diagnosis for all patients examined – constitute possible confounding variables. Other psychiatric disorders such as Borderline personality disorder are associated with empathy impairments as well (173). The inclusion of a psychiatric control group in study 2 might have added clarity in regard to the results. Follow-up studies with stricter inclusion criteria with respect to the patients’ group (preferably no comorbid psychiatric disorders) are warranted.

However, despite these limitations the findings of both studies contribute to the important research on the interplay of biological and psychological factors involved in substance addiction by expanding current knowledge on the role of testosterone in the withdrawal period of alcohol and opiate addiction as well as associated factors possibly relevant for the maintenance of these disorders.

4.6 Clinical implications

Especially the results of high testosterone levels during early withdrawal in both opiate and alcohol addiction as well as the finding of high personal distress in opiate-addicted patients have several diagnostic and therapeutic implications, provided they can be corroborated by further research.

According to the results presented, personal distress may play a role in the maintenance of opiate addiction, because opiates are able to alleviate the negative emotional states habitually evoked by high personal distress. Hence, interventions for opiate-addicted patients focusing on these specific empathy impairments could be developed. Similar suggestions have been made with respect to social cognition rehabilitation in alcohol addiction (101, 174, 175). Treatment programs could include psychoeducational information about personal distress and associated negative emotional states to improve the patients’ comprehension for
internal processes. Furthermore, emotion regulation abilities in regard to personal distress could be enhanced via tailored interventions. The opiate-addicted patients should learn and practice adequate coping strategies and emotion regulation skills in order to equip them with several alternatives to opiate use. Because the learned association between opiates and relief of negative emotional states is possibly very strong, repetition is crucial in regard to the training of new emotion regulation skills. When the patient masters the new skills, the latter should be practiced in high risk situations for opiate use, i.e. social situations with another person present who displays negative emotions. In these situations, dysphoric or anxious states are likely to be triggered in the patient because of high personal distress. Moreover, the treatment program should aim at strengthening self-regulation and diminishing impulsiveness so that the patient is capable to resist immediate impulses to use opiates instead of the new coping skills.

Further research is needed to illuminate the role of testosterone in regard to the maintenance of opiate and alcohol addiction. Nevertheless, the findings of altered testosterone levels during alcohol and opiate withdrawal suggest that testosterone and the HPG axis could be assumed fruitful new targets for the treatment of addiction. This has been proposed before (12, 137). Since study 1 implicates that alterations in testosterone levels during withdrawal are relevant in regard to craving, interventions aiming at the dysregulation of the HPG axis in addicted patients may be useful to prevent relapse. Provided that further research supports the hypotheses that high testosterone levels during early withdrawal and high personal distress are diagnostic markers for severe addiction, the assessment of testosterone levels during withdrawal and of personal distress as a trait could be used to distinguish severely from less severely addicted patients. In this case, patients with severe addiction could be identified faster and allocated in special treatment programs with interventions tailored for this patient group. In regard to personal distress, the Interpersonal Reactivity Index could be used as a diagnostic measure. However, it is important to note that further research is warranted to ensure that both high testosterone levels during early withdrawal as well as high personal distress as a trait are markers suitable for individual assessment of patients and not only applicable for the examination of groups.
4.7 Further research

Study 1 indicates a relevant interplay among testosterone, BDNF, and HPA axis activity in regard to alcohol craving during alcohol withdrawal. Further research is warranted to explore this interplay in more detail. As BDNF was previously reported to be associated with the symptomatology of opiate addiction (47, 61), further studies could investigate interactions between testosterone, BDNF, and HPA axis activity in opiate-addicted patients to explore whether similar associations between these factors influence opiate craving during opiate withdrawal. Like opiate addiction, alcohol addiction also seems to be associated with impairments in emotional empathy (101). Hence, it would be intriguing to examine a possible association of impaired emotional empathy with testosterone levels in alcohol-addicted patients, analogous to the one found in opiate-addicted patients.

In study 2, diacetylmorphine-maintained patients were examined. Further studies could investigate whether similar results regarding personal distress and testosterone levels during early withdrawal can be observed in patients being treated with other types of opiates as well. With respect to testosterone, similar results are to be expected: Previous research on testosterone levels in chronically opiate-using subjects typically reported lower levels compared to controls (26, 27), regardless of the type of opiate or whether the opiate was used to treat chronic pain (176) or for maintenance treatment (177).

As there are possible sex differences regarding the factors examined in both studies, further research with larger groups of female patients is needed to investigate whether the results reported in studies 1 and 2 are generalizable to female addicted patients. For example, there are sex differences regarding HPA axis activity and its response to psychological stress (178), the relationship between the HPG and the HPA axis (78), BDNF signaling (62), and the interplay of BDNF and testosterone (179). Moreover, a meta-analysis by Bawor and colleagues presented evidence that testosterone levels are affected differently in female opiate-addicted patients compared to male patients. The authors concluded that opiates may have sexually dimorphic endocrine disruptive mechanisms (26). Nevertheless, the number of studies investigating female opiate-addicted patients is still small and the results so far were mixed (26, 176, 180). Therefore, further research is warranted to clarify the role of testosterone in regard to female addicted patients. Future studies should preferably monitor the use of oral contraceptives as well as menstrual cycle phase. Because ovarian
hormones such as estradiol may as well contribute to sex differences in the prevalence and progression of substance use disorders (10), they should at best be assessed and possible associations with other variables such as HPA axis activity and BDNF could be examined. Prospective studies investigating testosterone levels during the whole withdrawal period and subsequently over a longer period of time after detoxification and examining associations with withdrawal symptoms, craving, and relapse behavior as well as BDNF and cortisol levels could help to improve our knowledge on the possible role of HPG axis dysregulation and the interplay among testosterone, BDNF, and HPA axis activity in regard to the maintenance of addiction, withdrawal symptomatology, and relapse after abstinence. Especially with respect to opiate addiction, further research on possible alterations of testosterone levels during the whole course of the withdrawal period is needed to corroborate the hypothesis that opiate-addicted patients display changes in testosterone levels during withdrawal similar to those reported for alcohol-addicted patients.

Another interesting line of possible further research concerns the influence of fetal testosterone on addiction. With respect to alcohol addiction, Lenz and colleagues proposed the early sex hormone activity model (12). According to this model, exposure to sex hormones in utero elicits organizational neuroadaptations which interact with the activational effects of sex hormones in adulthood and play an important role in the modulation of addictive behavior. There is some evidence supporting the assumption that high fetal testosterone is associated with alcohol addiction (12, 181, 182). Similar mechanisms may be hypothesized with respect to opiate addiction. Moreover, analogous to research concerning autistic traits (183), the influence of prenatal hormonal priming and possible organizational effects of fetal testosterone on empathy impairments in addicted patients could be investigated. Associations between 2D:4D ratio as a proxy for fetal testosterone with current testosterone levels before, during, and after withdrawal as well as with personal distress could be examined.

To clarify the association between personal distress and testosterone levels found in opiate-addicted patients during early withdrawal, study designs investigating this association in currently substance-using and in abstinent patients are necessary. Personal distress is a stable trait; therefore, it can be hypothesized that when examining chronically opiate-using and abstinent patients, higher scores in the personal distress scale compared to controls – analogous to the results when investigating patients during early withdrawal as in study 2 –
will be found. Studies examining possible associations of personal distress with craving and relapse behavior in addicted patients are needed to corroborate the assumption derived from the results of study 2 that high personal distress as a trait may be considered a factor relevant for the maintenance of opiate addiction. Furthermore, studies on the significance of high personal distress as a trait and high testosterone during early withdrawal as markers for severe addiction are warranted.
5. References


(40) Kuhn C. Emergence of sex differences in the development of substance use and abuse during adolescence. Pharmacol Ther 2015 Sep;153:55-78.


(50) Suliman S, Hemmings SMJ, Seedat S. Brain-derived neurotrophic factor (BDNF) protein levels in anxiety disorders: systematic review and meta-regression analysis. Front Integr Neurosci 2013 July 29;7:55.


Li M, Masugi-Tokita M, Takanami K, Yamada S, Kawata M. Testosterone has sublayer-specific effects on dendritic spine maturation mediated by BDNF and PSD-95 in pyramidal neurons in the hippocampus CA1 area. Brain Res 2012 Nov 12;1484:76-84.


Jacobsen JP, Mørk A. Chronic corticosterone decreases brain-derived neurotrophic factor (BDNF) mRNA and protein in the hippocampus, but not in the frontal cortex, of the rat. Brain Res 2006 Sep 19;1110(1):221-5.


Schaaf MJ, De Kloet ER, Vreugdenhil E. Corticosterone effects on BDNF expression in the hippocampus. Implications for memory formation. Stress 2000 May;3(3):201-8.


Littlefield AK, Sher KJ. The multiple, distinct ways that personality contributes to alcohol use disorders. Soc Personal Psychol Compass 2010 Sep;4(9):767-82.


Annex I

- Paper study 1: Association of testosterone and BDNF serum levels with craving during alcohol withdrawal

- Paper study 2: Positive association of personal distress with testosterone in opiate-addicted patients
Paper study 1: Association of testosterone and BDNF serum levels with craving during alcohol withdrawal

Contributions Katrin Stange:
KS contributed substantially to the interpretation of the data. Furthermore, she contributed significantly to the drafting of the article and the revision of the manuscript during the review process.

Authorization for use in dissertation received:
13 April 2017 (authorization by Lakshmi Priya, Sr Copyrights Coordinator, ELSEVIER).

Reference:
Association of testosterone and BDNF serum levels with craving during alcohol withdrawal

Annemarie Heberlein a, c, *, Bernd Lenz b, Birgitt Opfermann d, Michael Gröschl c, Eva Janke a, Katrin Stange a, Adrian Groh a, Johannes Kornhuber b, Helge Frieling a, Stefan Bleich a, Thomas Hillemacher a

*Center for Addiction Research (CARe), Department of Psychiatry, Social Psychiatry and Psychotherapy, Medical School Hanover, Germany
b Department of Psychiatry and Psychotherapy, Friedrich-Alexander University Erlangen-Nürnberg, Germany
c Bioanalytical Laboratory, Celerion Switzerland AG, Allmendstr. 32, 8320 Fehraltorf, Switzerland
d Medizinischer Dienst der Krankenversicherungen Niedersachsen, Hildesheimer Str. 265-267, 30519 Hannover, Germany

Article info

Article history:
Received 14 February 2016
Received in revised form 14 June 2016
Accepted 14 June 2016

Keywords:
Testosterone
Alcohol withdrawal
Alcohol dependence
BDNF
Abstinence

Abstract

Preclinical and clinical studies show associations between testosterone and brain-derived neurotrophic growth factor (BDNF) serum levels. BDNF and testosterone have been independently reported to influence alcohol consumption. Therefore, we aimed to investigate a possible interplay of testosterone and BDNF contributing to alcohol dependence. Regarding possible interplay of testosterone and BDNF and the activity of the hypothalamic pituitary axis (HPA), we included cortisol serum levels in our research. We investigated testosterone and BDNF serum levels in a sample of 99 male alcohol-dependent patients during alcohol withdrawal (day 1, 7, and 14) and compared them to a healthy male control group (n = 17). The testosterone serum levels were significantly (p < 0.001) higher in the patients' group than in the control group and decreased significantly during alcohol withdrawal (p < 0.001). The decrease of testosterone serum levels during alcohol withdrawal (days 1–7) was significantly associated with the BDNF serum levels (day 1: p = 0.008). In a subgroup of patients showing high cortisol serum levels (putatively mirroring high HPA activity), we found a significant association of BDNF and testosterone as well as with alcohol craving measured by the Obsessive and Compulsive Drinking Scale (OCDS). Our data suggest a possible association of BDNF and testosterone serum levels, which may be relevant for the symptomatology of alcohol dependence. Further studies are needed to clarify our results.

© 2016 Elsevier Inc. All rights reserved.

Introduction

Alterations in testosterone and brain-derived neurotrophic factor (BDNF) serum levels have been associated with alcohol consumption. For example, Etelälahi and colleagues reported a positive association between testosterone release and alcohol consumption in alcohol-prefering rats (Etelälahi, Saarikoski, & Eriksson, 2011). Preclinical data also show higher basal testosterone levels in alcohol-prefering rats compared to non-prefering rat lines (Apter & Eriksson, 2003), and link testosterone administration with alcohol intake (Lakoza & Barkov, 1980): in clinical studies a decrease of circulating testosterone levels related to duration of drinking has been observed, as well as a direct association between the amount of alcohol consumed per day and the amount of free testosterone serum levels (Shiels et al., 2009). Consistently, high estradiol and testosterone levels were found to be associated with alcohol consumption in boys (de Water, Braams, Crone, & Peper, 2013). Moreover, in adolescents, testosterone serum levels were reported to predict the amount of alcohol consumed in adulthood (Braams, Peper, van der Heide, Peters, & Crone, 2016). Concretely, high testosterone levels in younger males predicted high alcohol consumption in adult males, an association that the authors suggest to be attributable to neural reward response. While testosterone seems to affect alcohol drinking behavior, conversely, alcohol consumption is known to affect testosterone secretion (Ruusa & Bergman, 1996) in a dose-dependent fashion: in males, intake of lower ethanol doses (0.5 g/kg) was reported to increase testosterone levels 2 h after ingestion (Sarkola & Eriksson, 2003), whereas intake of higher...
ethanol dosages (1.5 g/kg) was reported to reduce testosterone release 10 h after ingestion (Välimäki et al., 1990).

BDNF serum levels were frequently reported to be decreased in alcohol-dependent patients compared to healthy controls (Huang et al., 2011; Zanardini et al., 2011). Moreover, high BDNF serum levels have been associated with withdrawal intensity during early alcohol withdrawal (Heberlein, Muschler, Wilhelm, et al., 2010; Huang et al., 2008). Further results suggest that BDNF serum levels may even be a possible marker of alcohol relapse probability. For example, Costa and colleagues presented data that indicate that high BDNF serum levels reduce the risk of relapse in alcohol-dependent patients (Costa, Girard, Dalmay, & Malauzat, 2011). Consistent with these results, we found an association between drinking history and promoter methylation of the BDNF gene in our own data: we found a negative association of BDNF IV-promoter methylation and duration of abstinence before relapse, which supports a possible protective effect of high BDNF expression regarding alcohol relapse (Heberlein et al., 2015). Further support for a possible association comes from animal data, which demonstrate that prolonged alcohol consumption results in a decrease of central BDNF expression (McCough et al., 2004; Raivo, Miettinen, & Kiiannaa, 2014), suggesting that the decrease of central BDNF expression resulting from alcohol consumption may promote further alcohol consumption.

Considering these study results, the question arises of a relevant interplay of testosterone and BDNF regarding alcohol intake. Indeed, there is research that supports a possible association between the neurotrophic growth factor BDNF and testosterone. For example, testosterone-dependent increase of hippocampal neuronal growth and testosterone-induced increase of synaptic plasticity were associated with concomitant BDNF expression (Atwi, McMahon, Scharfman, & Maclusky, 2016). Moreover, testosterone administration was shown to decrease cerebral ischemic infarct volume in rats by increasing BDNF expression in the affected area (Fanaei et al., 2014). Nevertheless, the concrete mechanisms of this possible interplay have not yet been elucidated. However, study results point toward an interaction between cytokine release, sex hormones, and neurotrophic growth factors. For example, Xu and colleagues reported that increased survival of bulbocavernous motoneurons by testosterone was blocked by tropomyosin receptor kinase B (TrkB), which is the high-affinity receptor for BDNF antagonists (Xu, Gingras, Bengston, Di Marco, & Forger, 2001). Conversely, there are study results which demonstrate interplay between cytokine release and the TrkB receptor, suggesting that neurotransmission via the TrkB may link inflammation, sex hormones, and neurotrophic growth factors (Zhang, Yao, & Hashimoto, 2016). Consistent with these reports are study results which suggest that the activity of the hypothalamic pituitary axis (HPA) (Moonat & Pandey, 2012) may be a relevant factor, which supports a possible association of BDNF and testosterone. Indeed, there are preclinical study results that demonstrate the influence of HPA activity on BDNF as well as on testosterone release. For example, basal HPA activity was negatively associated with cerebral BDNF expression. Moreover, a negative association between de novo synthesis of BDNF and the adaption of the stress response was reported (Magsoudi et al., 2014; Naert, Maurice, Tapia-Arancibia, & Givalois, 2007). Regarding a possible influence of HPA activity on testosterone levels, Toufexis and colleagues reported that physiological doses of dihydrotestosterone decreased basal levels of serum cortisol in male and female macaques and also decreased corticotrophin-releasing factor- (CRF) induced activation in male macaques (Toufexis & Wilson, 2012).

Study results also suggest a link between testosterone-related behavioral impulsivity (Cooper, Goings, Kim, & Wood, 2014) and HPA activity (Mehta, Welker, Zilioli, & Carré, 2015). Mehta et al. (2015) reported that risk taking and testosterone serum levels were positively associated in male and female probands when HPA activity was low, suggesting a link between impulsivity, testosterone release, and HPA activity. Partly closing the gap between alterations of testosterone levels, HPA activity, and alcohol consumption, prenatal exposure to ethanol was reported to reduce testosterone's effect on the HPA (Lan, Hellemans, Ellis, Viau, & Weinberg, 2009). Moreover, corticosterone replacement therapy was reported to result in increased alcohol consumption in adrenalectomized alcohol-prefering male rats, implicating a direct corticosterone effect on alcohol consumption in vulnerable populations (Fahlke & Eriksson, 2000).

These results suggest an association of high testosterone release, decreased HPA activity, increased BDNF expression, and behavioral traits, which may influence impulsive behavior such as alcohol consumption. Strengthening the hypothesis of a multilateral association of sex hormones, HPA activity, and BDNF, Franklin and colleagues reported that stress increased or decreased BDNF release, depending on the availability of sex hormones (Franklin & Perrot-Sinal, 2006).

The aim of our study was to investigate alterations of testosterone serum levels due to intoxication and during withdrawal in alcohol-dependent patients. Moreover, we focused on possible associations between the symptomatology of alcohol withdrawal, testosterone, and BDNF. Regarding the possible relevance of the activity of the HPA axis, we also investigated a possible association between testosterone and BDNF serum levels in subgroups of patients displaying high versus low cortisol levels.

Materials and methods

The present study was part of a large prospective research project (Studies in Neuroendocrinology and Neurogenetics in Alcoholism [NENA]) (Heberlein, Muschler, Lenz, et al., 2010) that was approved by the local Ethics Committee of the Friedrich-Alexander University Erlangen-Nürnberg. In this sample we had already investigated alterations of BDNF serum levels during alcohol withdrawal (Heberlein, Muschler, Wilhelm, et al., 2010). The investigation was conducted in accordance with the Declaration of Helsinki. Each participant gave written informed consent.

In total, we investigated the testosterone and BDNF serum levels of 99 male patients who suffered from alcohol dependence, according to ICD-10 and DSM-IV. All patients were admitted for detoxification treatment (Klinik für Psychiatrie, Psychotherapie und Psychosomatik, Bezirksklinikum Obermain, Kutzenberg, Germany). The patients’ group consisted of smokers and non-smokers (10 non-smokers, 81 smokers, 6 former smokers, 2 unknown). Table 1 shows the demographic data of the patients’ group. Patients with concomitant psychiatric illnesses, substance abuse apart from alcohol or nicotine, existence of severe somatic illnesses (in particular patients suffering from any type of cancer), known autoimmune diseases, or known HPA axis deregulations were not enrolled in the study. In addition, patients with a positive history of cerebral damage (e.g., ischemia or cerebral hemorrhage) were excluded. All patients underwent a detailed physical examination, routine laboratory testing, and urine drug screening. Patients received carbamazepine and/or clomethiazole in order to treat alcohol-withdrawal symptomatology. Dosages were adjusted to the individual severity of alcohol withdrawal. Blood samples were taken before the patients took their morning medication.

Breath alcohol concentration was measured on admission and during alcohol withdrawal using the alcohol breath analyzer (Draeger, Dietikon, CH). Additional data such as age, body mass index (BMI), years of drinking, and daily intake of alcohol in grams
were obtained by interview. Data regarding affective symptoms were collected by the Beck’s Depression Inventory (BDI) (Beck, Ward, Mendelson, Mock, & Erbaugh, 1961) and the State and Trait Anxiety Inventory (STAI-I and STAI-II) (Spielberger, Gorsuch, & Lushene, 1970). Intensity of alcohol craving was measured by the Obsessive and Compulsive Drinking Scale (OCDS) (Anton, Moak, & Latham, 1995). Severity of alcohol withdrawal was measured by the Clinical Institute Withdrawal Assessment for Alcohol (CIWA-A) (Latham, 1995). Severity of alcohol dependence was assessed by the Severity of Alcohol Dependence (SESA) questionnaire measure (Fiore et al., 2009). A. Heberlein et al. / Alcohol 54 (2016) 67–72

The BDNF, the testosterone, and the cortisol serum levels were assessed using enzyme-linked immunosorbent assay (ELISA) (DY248, KGE010, KA1885, R&D Systems, Wiesbaden-Nordenstadt, Germany). All the assays were performed according to the manufacturer’s directions. The lower thresholds of determination were 23.4 pg/mL (BDNF), 0.041 ng/mL (testosterone), and 0.456 ng/mL (cortisol). The assay ranges were 23.4–1500 pg/mL (BDNF), 0–10 ng/mL (testosterone), and 0.66–20 ng/mL (cortisol). The interassay coefficients of variation were 5.0 and 8.7%. Besides BDNF, testosterone, and cortisol serum levels, we assessed liver enzyme levels, creatinine, urea blood count, and c-reactive peptide levels in all patients and controls.

Statistical analysis

The testosterone serum levels were not normally distributed according to the Kolmogorov–Smirnov test. Log- and In-transformation did not transform the testosterone serum levels into normal distribution; therefore, non-parametric statistics were applied. In order to assess the alterations of testosterone serum levels throughout alcohol withdrawal, three difference values were calculated, which mirror the alterations of testosterone serum levels from day 1 to day 7, from day 7 to day 14, and from day 1 to day 14. Moreover, in order to assess a possible impact of HPA activity, a median split was calculated to diverge patients with high (subgroup A) and low (subgroup B) cortisol serum levels throughout alcohol withdrawal.

Correlations were calculated by the Spearman’s correlation coefficient. Mean differences between the testosterone serum levels of the alcohol-dependent patients on day 1, 7, and 14 were calculated by Friedman test. Group-to-group differences

---

**Table 1**

Demographic data of the patients’ and the control group, means, and standard deviation (SD) of the test results.

<table>
<thead>
<tr>
<th></th>
<th>Patients’ group</th>
<th>Control group</th>
<th>p</th>
<th>Early abstinent (n = 24)</th>
<th>Positive breath alcohol (n = 57)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>42.22 ± 7.83</td>
<td>44.41 ± 9.63</td>
<td>n.s.</td>
<td>42.50 ± 10.64</td>
<td>43.96 ± 7.13</td>
<td>n.s.</td>
</tr>
<tr>
<td>BMI</td>
<td>24.62 ± 3.60</td>
<td>26.44 ± 4.58</td>
<td>n.s.</td>
<td>25.01 ± 5.49</td>
<td>25.07 ± 4.02</td>
<td>n.s.</td>
</tr>
<tr>
<td>Years of drinking (y)</td>
<td>9.4 ± 7.67</td>
<td>n.a.</td>
<td>n.s.</td>
<td>8.95 ± 9.26</td>
<td>8.74 ± 7.22</td>
<td>n.s.</td>
</tr>
<tr>
<td>Daily intake (g)</td>
<td>194.90 ± 83.42</td>
<td>n.a.</td>
<td>n.s.</td>
<td>171.55 ± 81.90</td>
<td>202.02 ± 88.63</td>
<td>n.s.</td>
</tr>
<tr>
<td>BDNF (pg/mL)</td>
<td>Day 1: 641.67 ± 511.71</td>
<td>728.84 ± 383.38</td>
<td>n.s.</td>
<td>Day 1: 478.55 ± 350.14</td>
<td>Day 1: 706.24 ± 512.05</td>
<td>Day 1: 0.044</td>
</tr>
<tr>
<td></td>
<td>Day 7: 556.33 ± 400.92</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Day 7: 527.98 ± 439.09</td>
<td>Day 7: 551.71 ± 420.39</td>
<td>Day 7: n.s.</td>
</tr>
<tr>
<td>Testosterone (ng/mL)</td>
<td>Day 1: 10.05 ± 11.42</td>
<td>5.11 ± 5.42</td>
<td>Day 1: -0.001</td>
<td>Day 1: 14.19 ± 16.88</td>
<td>Day 1: 11.02 ± 6.68</td>
<td>Day 1: n.s.</td>
</tr>
<tr>
<td>Cortisol (nmol/L)</td>
<td>Day 1: 5.64 ± 8.69</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Day 1: 9.87 ± 6.16</td>
<td>Day 1: 8.41 ± 3.86</td>
<td>Day 1: n.s.</td>
</tr>
<tr>
<td></td>
<td>Day 7: 5.64 ± 101.22</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Day 7: 164.39 ± 91.74</td>
<td>Day 7: 221.42 ± 105.01</td>
<td>Day 7: 0.035</td>
</tr>
<tr>
<td>OCDS total score</td>
<td>Day 1: 16.1 ± 18.02</td>
<td>8.90 ± 5.41</td>
<td>Day 1: 16.88 ± 6.3</td>
<td>Day 1: 11.34 ± 7.12</td>
<td>Day 1: 14.05 ± 7.01</td>
<td>Day 1: n.s.</td>
</tr>
<tr>
<td>cortisol</td>
<td>CIWA</td>
<td>Day 1: 15.64 ± 4.23</td>
<td>n.a.</td>
<td>Day 1: 14.04 ± 3.5</td>
<td>Day 1: 15.08 ± 4.27</td>
<td>Day 1: 0.045</td>
</tr>
<tr>
<td></td>
<td>Day 7: 12.84 ± 2.75</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Day 7: 12.73 ± 2.35</td>
<td>Day 7: 12.93 ± 2.87</td>
<td>Day 7: n.s.</td>
</tr>
<tr>
<td>BDI</td>
<td>Day 14: 12.33 ± 2.35</td>
<td>3.00 ± 3.77</td>
<td>Day 1: -0.001</td>
<td>Day 1: 17.96 ± 10.08</td>
<td>Day 1: 17.62 ± 9.96</td>
<td>Day 1: n.s.</td>
</tr>
<tr>
<td></td>
<td>Day 1: 4.87 ± 12.59</td>
<td>31.47 ± 5.34</td>
<td>Day 1: -0.001</td>
<td>Day 1: 46.96 ± 9.54</td>
<td>Day 1: 49.05 ± 13.36</td>
<td>Day 1: n.s.</td>
</tr>
<tr>
<td>STAI-II</td>
<td>Day 7: 36.40 ± 10.96</td>
<td>14.07 ± 6.94</td>
<td>Day 7: 0.006</td>
<td>Day 7: 35.09 ± 12.9</td>
<td>Day 7: 38.27 ± 10.64</td>
<td>Day 7: n.s.</td>
</tr>
<tr>
<td>STAI-II</td>
<td>Day 1: 4.78 ± 10.83</td>
<td>30.65 ± 6.94</td>
<td>Day 1: -0.001</td>
<td>Day 1: 49.0 ± 11.32</td>
<td>Day 1: 47.38 ± 10.89</td>
<td>Day 1: n.s.</td>
</tr>
<tr>
<td>SESa</td>
<td>Day 1: 48.92 ± 18.05</td>
<td>n.a.</td>
<td>n.a.</td>
<td>Day 1: 44.70 ± 18.17</td>
<td>Day 1: 49.94 ± 20.36</td>
<td>Day 1: n.s.</td>
</tr>
</tbody>
</table>

n.s.: not significant; n.a.: not available.
between the healthy control group and the patients’ group were calculated by the Mann–Whitney U test. Significance level was set to \( \alpha = 0.05 \).

Data were analyzed by SPSS 20 (SPSS Inc., Chicago, IL). Graphs were developed by GraphPad Prism™ 5.0 (GraphPad Software, Inc., San Diego, CA).

**Results**

**Demographic data of the patients’ group**

Data concerning the demographic characteristics of both groups and data concerning the history and amount of alcohol consumption in the patients’ group are shown in Table 1. There was no association between the testosterone serum levels and the BMI of the participants. Testosterone levels on day 14 of alcohol withdrawal were significantly associated with the age of the participants (\( \rho = -0.265, p = 0.012 \)).

**Association of testosterone with liver enzyme levels and blood count**

The testosterone serum levels were not associated with liver enzyme levels during alcohol withdrawal (data not shown). There was no association with leukocyte count, thrombocyte count, or the testosterone serum levels during alcohol withdrawal (data not shown).

**Association of testosterone serum levels and alcohol withdrawal medication**

There was a significant positive association between clomethiazole dosages and testosterone serum levels on day 1 of alcohol withdrawal in patients showing positive breath alcohol concentrations on admission (\( \rho = 0.358, p = 0.010 \)). In subgroup A, testosterone serum levels on day 1 were significantly associated with clomethiazole dosage as well (\( \rho = 0.588, p < 0.001 \)).

**Testosterone serum levels in alcohol-dependent patients compared with healthy controls**

Serum levels of testosterone were significantly higher in the alcohol-dependent patients compared with healthy controls (day 1: \( U = -3.652, p < 0.001 \), day 7: \( U = 3.238, p = 0.001 \), day 14: \( U = 3.019, p = 0.003 \); see Fig. 1). Testosterone serum levels decreased significantly during alcohol withdrawal (\( \chi^2 = 32.23, p < 0.001 \), see Fig. 1).

The BDNF serum levels of the experimental group were not significantly altered compared with the levels of the healthy control group (Heberlein, Muschler, Wilhelm, et al., 2010). The BDNF serum level decreased slightly but not significantly during alcohol withdrawal (data not shown).

**Association between testosterone serum levels and alcohol consumption**

**Association with breath alcohol concentration**

There was no association between breath alcohol concentration and the BDNF, cortisol, and testosterone serum levels in the entire patients’ group.

There was a significant trend toward higher BDNF serum levels in patients showing positive alcohol breath levels at admission (\( n = 57 \)) compared to early abstinent patients (\( n = 24, p = 0.044 \)). Apart from that, there were no significant differences regarding psychometrics and serum levels between early abstinent and alcohol-intoxicated patients (see Table 1 for details).

In those patients showing higher HPA activity (subgroup A, 29 patients), the testosterone serum levels were significantly associated with breath alcohol concentration on day 1 of alcohol withdrawal (\( \rho = -0.508, p = 0.005 \)). Moreover, the decrease of testosterone serum levels from day 1 to day 7 was significantly associated with breath alcohol concentration (\( \rho = 0.526, p = 0.004 \)).

**Association with anamnesis of alcohol consumption**

There was no association between the duration of alcohol consumption or the daily alcohol intake and the testosterone serum levels in the entire patients’ group. Nevertheless, in the group of patients showing high HPA activity (subgroup A), the testosterone serum levels on day 14 were significantly associated with the duration of self-reported alcohol consumption (\( \rho = -0.507, p = 0.001 \)).

**Association of testosterone, BDNF, and cortisol serum levels**

Testosterone serum levels were significantly different in subgroup A and subgroup B: testosterone serum levels were significantly increased in those patients showing higher cortisol serum levels on day 1 (Mann–Whitney U \( Z = -2.58, p = 0.010 \)).

Testosterone serum levels were significantly associated with cortisol serum levels on day 1 (\( \rho = 0.243, p = 0.017 \)) and day 14 of alcohol withdrawal (\( \rho = 0.366, p < 0.001 \)).

The decrease of testosterone serum levels from day 1 to day 7 of alcohol withdrawal was significantly associated with the BDNF serum levels on day 1 (\( \rho = -0.268, p = 0.008 \)).

**Association of testosterone serum levels and the severity of alcohol dependence**

The decrease of testosterone serum levels from day 1 to day 14 was significantly associated with the XTU subscale of the SESAscale (\( \rho = 0.227, p = 0.026 \)). There was an association neither with the further SESA subscales nor with the total value of the SESAscale (data not shown).

**Association of testosterone serum levels with the symptomatology of alcohol withdrawal**

The decrease of the testosterone serum levels from day 1 to day 7 was significantly associated with alcohol craving measured by the OCDS (\( \rho = 0.248, p = 0.014 \) on day 7 and with the compulsive subscale of the OCDS on day 7 (\( \rho = 0.324, p = 0.001 \)).
Moreover, in subgroup A the decrease of testosterone serum levels from day 1 to day 7 and from day 7 to day 14 was significantly associated with BDNF serum levels (rho = −0.369, p = 0.013) and with compulsive alcohol craving on day 7 of alcohol withdrawal (rho = 0.417, p = 0.004). Conversely, the BDNF serum levels showed a significant association with the OCDS total score (rho = 0.429, p = 0.003), the obsessive subscale (rho = 0.352, p = 0.018), and the compulsive subscale (rho = 0.383, p = 0.009) in this subgroup on day 1.

There was no association between the testosterone serum levels and the severity of the alcohol withdrawal syndrome measured by the CIWA score (data not shown). There was no significant association between the testosterone serum levels, the decrease of the testosterone serum levels, and the BDI score or the STAI-I/STAI-II scores (data not shown).

Discussion

In this study, we investigated a possible link between alterations in testosterone, BDNF, and cortisol serum levels and alcohol consumption in a sample of male alcohol-dependent patients.

While study results typically demonstrate a decrease of testosterone levels due to chronic alcohol consumption (Maneesh, Dutta, Chakrabarti, & Vasudevan, 2006), higher testosterone levels have been reported in male alcohol-dependent patients in the first 3 months of abstinence (Hasselblatt, Kriegl-Hartig, Hüfner, Halaris, & Ehrenreich, 2003). Consistent with such reports, we found higher testosterone serum levels in the patients’ group compared to the healthy control group. High testosterone levels could be explained by dysregulation of testosterone release due to alcohol withdrawal. Consistently, we found high variations of testosterone serum levels in our study (see Table 1 for details) as well as a positive association between cortisol serum levels and testosterone serum levels on day 1 and day 14 of alcohol withdrawal.

We also found a positive association between the clomethiazole dosages and the testosterone serum levels. This indicates that the high testosterone serum levels observed in our sample may at least partly be explained by alcohol-withdrawal medication.

In agreement with study results supporting a suppressive effect of alcohol consumption on testosterone serum levels (Forquer, Hashimoto, Roberts, & Wiren, 2011), we found a negative association between the testosterone serum levels and initial breath alcohol concentration in subgroup A (those patients showing high HPA activity). Furthermore, we found a negative association between the testosterone serum levels and drinking history after early alcohol withdrawal (on day 14) as well as a positive association between the decrease of testosterone serum levels from day 1 to day 14 of alcohol withdrawal and initial alcohol intoxication in subgroup A, which is consistent with study results reporting decreased testosterone serum levels due to chronic alcohol consumption (Maneesh et al., 2006).

Regarding the symptomatology of alcohol withdrawal, our data show a positive association between the decline of testosterone serum levels during alcohol withdrawal, the BDNF serum levels, and the intensity of alcohol craving in early alcohol withdrawal. We also found a negative association between the decrease of testosterone serum levels and the BDNF serum levels, which were positively associated with the intensity of alcohol craving measured by the OCDS in subgroup A of our sample. These results suggest that there may be a relevant association between HPA activity, testosterone, and BDNF levels as reported before by some authors (Franklin, Zou, Yu, & Costello, 2006). Our results support the assumption that HPA activation is involved in the interplay between testosterone and BDNF.

Earlier study results showed negative associations between HPA activity, testosterone (Mehta et al., 2015), and BDNF (Franklin & Perrot-Sinal, 2006) release, as well as positive associations between testosterone and BDNF (Fanaei et al., 2014). Such results indicate that high HPA activity interferes with neuroregeneration and neuroneogenesis (Allen, Purves-Tyson, Fung, & Shannon Weickert, 2015), which are typically stressed following loss of hippocampal volume in depressive disorders (Yun et al., 2016). Moreover, animal data suggest that corticosteroids trigger alcohol consumption in vulnerable populations (Fahlke & Eriksson, 2000). We found a negative association between the decrease of the testosterone serum levels during alcohol withdrawal and BDNF serum levels, a result that does not fit easily with a possible positive association of BDNF and testosterone reported earlier (Yun et al., 2016). It is likely that alcohol withdrawal and alcohol-withdrawal medication may explain the results presented here. Indeed, there are preclinical study results that support the hypothesis that ethanol consumption may alter the relationship between testosterone and the HPA (Zhang et al., 2016). Therefore, it seems likely that the alcohol craving associated with alcohol withdrawal is explained by the association of BDNF and testosterone. Further studies will be necessary to clarify the implications of the associations found in our sample.

Although our data are not sufficient to allow causal conclusions, they support earlier research which suggests an interplay of sex hormones and BDNF (Fanaei et al., 2014), possibly relevant for the symptomatology of alcohol dependence. Concretely, our data are consistent with earlier results presented by our group, which demonstrate that BDNF is associated with the symptomatology of alcohol withdrawal and alcohol relapse (Heberlein et al., 2014, 2015). The results obtained in this study regarding a possible association between testosterone serum levels and BDNF serum levels supplement earlier results of our group that show a negative association between BDNF serum levels and serum levels of the cytokine tumor necrosis factor a (TNF-a). Taking into account animal studies that demonstrate negative associations between testosterone and TNF-a (Bini et al., 2015), one could speculate about a complex interplay of different peptides such as cytokines, sex hormones, etc., and BDNF, which may contribute to the symptomatology of alcohol dependence.

Our study suffers from several limitations that constrain the interpretability of our results. One major limitation is due to the non-linearity of our testosterone data. This prompted us to use non-parametric statistics instead of variance analysis, which would have allowed the inclusion of possible covariates. Moreover, our samples were heterogeneous regarding smoking status, which may have influenced our results. A further restriction is the correlating character of our research as well as the single daily measurement of the testosterone and BDNF serum levels. A prospective study setting measuring cortisol, BDNF, and testosterone throughout the day and investigating more clearly the association between stress regulation and BDNF-testosterone interaction as suggested by our data would clearly have improved the clarity of our results.

Summing up, our study supports earlier research regarding a possible interaction between testosterone and BDNF. Moreover, it enriches current knowledge by supporting the hypothesis of a possible interplay between testosterone and BDNF, presumably dependent on HPA activity. According to our results, the decrease of testosterone release during alcohol withdrawal may underlie alcohol craving by interaction with BDNF expression. Therefore, it seems likely that testosterone contributes to core symptoms of alcohol dependence, such as alcohol craving. Further research is warranted in order to unravel the causalities of the associations observed. It is necessary to regard the various limitations of our study as well as its correlating character in order to confirm our results.
References


Paper study 2: Positive association of personal distress with testosterone in opiate-addicted patients

Contributions Katrin Stange:
KS drafted the first version of the study design and contributed most prominently to the final version of the conception and design of the study. She was fully responsible for data acquisition. Moreover, KS analyzed the data and contributed most prominently to the interpretation of the data. She wrote the first draft of the article and revised the manuscript throughout the review process.

Authorization for use in dissertation received:
8 March 2017 (Author Publishing Agreement, Taylor & Francis).

Reference:
Positive association of personal distress with testosterone in opiate-addicted patients

Katrin Stange, Mathias Krüger, Eva Janke, Ralf Lichtinghagen, Stefan Bleich, Thomas Hillemacher & Annemarie Heberlein

To cite this article: Katrin Stange, Mathias Krüger, Eva Janke, Ralf Lichtinghagen, Stefan Bleich, Thomas Hillemacher & Annemarie Heberlein (2017) Positive association of personal distress with testosterone in opiate-addicted patients, Journal of Addictive Diseases, 36:3, 167-174, DOI: 10.1080/10550887.2017.1303980

To link to this article: http://dx.doi.org/10.1080/10550887.2017.1303980
ARTICLE

Positive association of personal distress with testosterone in opiate-addicted patients

Katrin Stange, MS a, Mathias Krüger, MS b, Eva Janke, MD c, Ralf Lichtinghagen, PhD c, Stefan Bleich, MD a, Thomas Hillelacher, MD c, and Annemarie Heberlein, MD a

a Center for Addiction Research (CARE), Department of Psychiatry, Social Psychiatry and Psychotherapy, Hanover Medical School, Germany; b Department of Psychology, University of Bonn, Germany; c Institute for Clinical Chemistry, Hanover Medical School, Germany

ABSTRACT
Clinical studies report that substance addictions are associated with sociocognitive impairments. Regarding opiate-addicted patients, the few existing studies point to deficits in empathic abilities. Previous research suggests that testosterone might be a relevant biomarker of these impairments. The authors aimed to investigate whether opiate-addicted patients show specific impairments in emotional (empathic concern, personal distress) and cognitive empathy compared to healthy controls. Furthermore, the authors aimed to assess possible associations of testosterone levels with impaired empathic abilities in the patients’ group. In this cross-sectional study, 27 opiate-addicted, diacetylmorphine-maintained patients (21 males, age mean 41.67 years, standard deviation 8.814) and 31 healthy controls (23 males, age mean 40.77 years, standard deviation 8.401) matched in age, sex, and educational level were examined. Cognitive and emotional empathy were measured via the German version of the Interpersonal Reactivity Index and salivary testosterone levels were assessed. The authors found higher personal distress scores (p < 0.01, d = 0.817) and higher testosterone (p < 0.001, d = 1.093) in the patients’ group compared to controls. Moreover, a positive correlation was found between testosterone and personal distress among the patients’ group (r = 0.399, p < 0.05). Opiate-addicted patients show specific impairments in emotional empathy, namely higher personal distress, which has clinical implications regarding social cognition rehabilitation and relapse prevention. The current data point toward testosterone as a possible biomarker for these sociocognitive impairments and suggest that high personal distress and high testosterone during withdrawal are possible markers for severe opiate addiction.

KEYWORDS
Testosterone; personal distress; opiate addiction; empathy

Introduction
Clinical studies reported that substance addictions are associated with impairments in several domains of social cognition such as emotional empathy, 1 theory of mind, 2 and decoding of emotions, 3 resulting in difficulties in the interpretation of social situations and maladaptive behavior in social interactions. Sociocognitive abilities even seem to be relevant regarding the severity of substance use and addiction. For example, Raisjouyan and colleagues 4 found a significant negative correlation between emotional intelligence obtained via self-report and the number of relapses in a group of opiate, amphetamine, or alcohol-addicted patients. Kun and Demetrovics 5 reported that a lower level of emotional intelligence is associated with more intensive smoking, alcohol use, and illicit drug use.

Regarding opiate-addicted patients, it is not clear yet which domains of social cognition show specific deviations compared to healthy controls. A promising line of research focuses on the investigation of possible impairments in empathy. Empathy is crucial for social functioning and prosocial behavior. 6 It is a multidimensional concept with two different facets. 7 Cognitive empathy refers to the ability to infer the mental state of another person. Emotional empathy refers to one’s own affective reactions when perceiving the emotions of another person, namely empathic concern, associated with sympathy and compassion, or personal distress, a self-centered reaction associated with an aversive state of over-arousal. 8 This line of research is especially important in terms of relapse prevention, as difficulties in social interactions...
constitute a major motive for opiate use in addicted patients.\textsuperscript{9,10} A profile of the specific deficits in empathic abilities will help to develop new rehabilitative programs and psychotherapeutic methods specifically targeting the impaired sociocognitive domains.

The few existing studies using a behavioral paradigm to assess cognitive empathy found evidence for deficits in opiate-addicted patients. McDonald and colleagues\textsuperscript{11} reported an impaired capacity for emotion perception in methadone- or buprenorphine-maintained patients compared to controls and abstinent opiate-addicted patients. Accordingly, Kornreich and colleagues\textsuperscript{12} reported impairments in the decoding of emotional facial expressions in recently detoxified opiate-addicted patients. Ferrari and colleagues\textsuperscript{13} found lower scores in the factor emotional empathy of the empathy quotient in a group of polysubstance users that included opiate-addicted patients compared to controls. Tomei and colleagues\textsuperscript{14} found lower self-reported empathic concern and higher personal distress in methadone-maintained patients compared to healthy controls.

So far, little is known about the physiological correlates and potential biomarkers of possible impairments in empathy. However, knowledge of low-level biological components that underlie social cognition deficits in addicted patients may generate promising routes for social cognition rehabilitation. Previous research points to the sex hormone testosterone as a possible candidate. Study results regarding nonclinical populations suggest a down-regulation of social intelligence induced by the hormone. On the one hand, testosterone seems to have an effect on an organizational level by influencing fetal brain development. For example, Chapman and colleagues\textsuperscript{15} found a negative correlation between fetal testosterone and empathy in children. On the other hand, testosterone has specific activational effects which negatively influence current performance. Testosterone application studies reported impairments in emotional as well as cognitive empathy. Hermans and colleagues\textsuperscript{16} found impairments in facial mimicry after the application of the hormone. Van Honk and colleagues\textsuperscript{17} reported impairments in cognitive empathy assessed by the Reading the Mind in the Eyes test after the application of testosterone. This effect was predicted by second-to-fourth digit (2D:4D) ratio, a proxy of fetal testosterone. The group argued that early neurodevelopmental effects of testosterone might facilitate the activational effects of this hormone in adulthood, influencing current performance.

Opiate addiction occurs more often in males than in females.\textsuperscript{18} Because of the role of sex hormones in central nervous system regulation, they qualify as a possible biological basis for this disparity and an involvement in the neurobiology of opiate addiction is suggested.\textsuperscript{19} Regarding testosterone, men display higher levels than women\textsuperscript{20,21} which—considering the sex differences in prevalence rates—indicates a possible positive association between this hormone and opiate addiction. However, previous studies found lower testosterone levels in patients with chronic opiate use compared to healthy controls,\textsuperscript{22,23} but results of other groups were mixed.\textsuperscript{24,25} For example, Bliesener and colleagues\textsuperscript{24} reported that the testosterone levels of buprenorphine-maintained patients did not differ from those of healthy controls and were significantly higher than the testosterone levels of methadone-maintained patients. Moreover, testosterone is linked with impulsivity.\textsuperscript{26,27} This supports the hypothesis of a positive association between testosterone and opiate addiction, because the association of impulsivity and substance use disorders is well documented, in particular regarding opiate use.\textsuperscript{28,29} High impulsivity seems to be both a risk factor for substance addictions and a consequence of prolonged drug use.\textsuperscript{30,31} Studies regarding alcohol report analogous associations of alcohol consumption with testosterone,\textsuperscript{32} as well as impulsivity.\textsuperscript{33}

To the best of the authors’ knowledge, this is the first attempt to investigate associations of empathic abilities with current levels of testosterone as a potential biomarker of sociocognitive deficits in opiate-addicted patients. The authors aimed to examine whether patients with severe opiate addiction show impairments in (1) cognitive and (2) emotional empathy in comparison to healthy controls. Based on the results of previous studies, the authors expected (1) lower cognitive empathy in the patients’ group compared to controls. With regard to (2), the authors hypothesized that opiate-addicted patients show a specific pattern of deviations in emotional empathy with lower empathic concern and higher personal distress compared to controls. Furthermore, the authors aimed to (3) assess current testosterone levels in opiate-
addicted patients in comparison to healthy controls and (4) explore possible associations with empathic abilities among the patients’ group.

Methods
Sample
The authors conducted a cross-sectional study comparing 27 patients (21 males; age range 27–57 years, median 42, mean 41.67, standard deviation 8.814) who fulfilled the diagnostic criteria of opiate addiction according to International Classification of Diseases, 10th Revision (ICD-10) and Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV) to 31 healthy controls (23 males; age range 24–55 years, median 41, mean 40.77, standard deviation 8.401). All patients participated in the structured diacetylmorphine maintenance program of the Hanover Medical School and received individual doses of injectable diacetylmorphine once or twice per day. This program is designed for severely and long-term addicted patients who have failed conventional maintenance treatment with methadone or buprenorphine in the past. The sample includes patients with comorbid substance addictions and other psychiatric disorders, as well as health problems which is highly typical for this population. For all patients, opiate addiction was the principal diagnosis. There was no major psychiatric comorbidity (i.e., no patients with acute psychosis, acute manic episode, or severe depression). Patients with human immunodeficiency virus (HIV)-infections or with positive breath alcohol concentrations were excluded from the study. One patient was excluded because he was not able to answer the interviewer’s questions and fill out the self-report questionnaire due to intoxication.

Controls were recruited from the community and matched in age, sex, and educational level to the opiate-addicted patients to address potential sources of bias. Controls did not suffer from any current somatic disease. They were examined regarding psychiatric disorders using the Structured Clinical Interview for DSM-IV
text
and were negative for all Axis-I psychiatric disorders according to ICD-10 and DSM-IV. They were not treated with any psychopharmacological medication or psychotherapy. The study was approved by the ethics committee of the Hanover Medical School. All participants gave written informed consent.

Procedure
The salivary testosterone samples were taken between 8:30 a.m. and 10:30 a.m. with a time frame of 20 min after each sample. In the patients’ group, the first testosterone sample was taken directly before the patients received their regular doses of diacetylmorphine. After obtaining the second testosterone sample, demographic data were collected. Afterward, empathy was measured.

Measures
The five salivary testosterone samples of each participant were pooled. Testosterone levels were assessed using enzyme-linked immunosorbent assay (ELISA). Demographic data were obtained by interview and self-report. Empathy was assessed with the German version of the Interpersonal Reactivity Index, the Saarbrücker Persönlichkeitsfragebogen (SPF). This self-report questionnaire measures two facets of empathy: cognitive empathy (perspective taking) and emotional empathy (empathic concern, personal distress, and fantasy). The perspective taking scale measures the ability to interpret a situation according to the inferred perspective of another person. The subscale empathic concern assesses the tendency to feel compassion toward another person. The subscale personal distress measures the tendency to experience negative emotions when another person in need is present. The fantasy scale assesses the tendency to identify with fictional protagonists.

Data analysis
An exploratory factor analysis (maximum likelihood extraction method, varimax rotation) was performed on the whole sample to verify conformity with the original dimensions of the Interpersonal Reactivity Index. The Kaiser-Meyer-Olkin (KMO) test was calculated to confirm adequacy of the sample. Cattell’s scree plot was used to determine the number of factors. To assess reliabilities, the authors calculated Cronbach’s alpha. The matching of the control group to the patients’ group was tested by the t-test for independent samples and Pearson’s Chi-squared test. Group differences between the healthy control group and the patients’ group were calculated by the t-test for independent samples (one-tailed for cognitive and emotional empathy, two-tailed for testosterone).
Significance level was set to 0.05. Using the Levene test, the authors checked the homogeneity of the variances. Because the variances were not homogenous for testosterone, the Welch $t$-test was used to calculate the group differences regarding this variable. In order to establish effect sizes, Cohen’s $d$ was calculated. Correlations were computed using Pearson’s correlation coefficient (all two-tailed). The authors also conducted partial correlations. There was no missing data. All calculations were computed using SPSS 24.

**Results**

**Preliminary analyses**

Because the authors did not formulate any hypotheses concerning the fantasy scale of the SPF, only the items belonging to the remaining scales (perspective taking, empathic concern, and personal distress) were included in the factor analysis. The KMO test confirmed adequacy of the sample ($KMO = 0.706$). Cattell’s scree plot pointed toward three factors. 62.33% of the total variance was explained. The original dimensions were confirmed (perspective taking: items 4, 10, 14, 16; empathic concern: items 1, 5, 11; personal distress: items 3, 6, 8, 13). One item (9) of the empathic concern subscale was eliminated because it was loading too high on factor one (perspective taking) and because of its correspondence to the perspective taking scale as well as the fantasy scale in terms of content.

The reliabilities of the dimensions generated by the factor analysis were then tested through Cronbach’s alpha tests. The reliability of the perspective taking scale (items 4, 10, 14, 16) was good ($\alpha = 0.805$). The empathic concern subscale (items 1, 5, 11; $\alpha = 0.698$) and the personal distress subscale (items 3, 6, 8, 13; $\alpha = 0.675$) had acceptable reliabilities. All items had acceptable to high item-scale-correlations. Subsequently, aggregated scores for each scale (perspective taking: items 4, 10, 14, 16; empathic concern: items 1, 5, 11; personal distress: items 3, 6, 8, 13) were computed which then became the measures for hypothesis testing.

**Group differences regarding empathy and testosterone between the patients’ group and the control group**

Regarding the personal distress scale of the SPF, the authors found higher scores in the patients’ group compared to the control group with a large effect size ($t = 3.100, p < 0.01, df = 56, d = 0.817$). The authors found higher testosterone levels in the patients’ group compared to the healthy control group with a large effect size as well ($t = 3.955, p < 0.001, df = 34.006, d = 1.093$).

**Association of testosterone and empathy among the patients’ group**

A significant positive correlation of testosterone and personal distress was found among the patients’ group ($r = 0.399, p < 0.05, n = 27$). Looking at the whole sample, hepatitis ($r = 0.532, p < 0.001, n = 58$) and smoking status ($r = 0.325, p < 0.05, n = 58$) correlated with testosterone. Hepatitis correlated with personal distress in the whole sample as well ($r = 0.303, p < 0.05, n = 58$). Therefore, a partial correlation of testosterone and personal distress was conducted among the patients’ group with hepatitis and smoking status as control variables. The positive correlation between testosterone and personal distress remained significant ($r = 0.434, p < 0.05, n = 27, df = 23$).

**Complementary results**

As the number of female participants differed in both groups and because there are natural sex differences regarding testosterone levels in men and women, the authors conducted group analyses regarding male patients ($n = 21$) and controls ($n = 23$) only. The authors found analogous group differences regarding personal distress ($t = 3.752, p < 0.001, df = 42$) as well as testosterone ($t = 4.068, p < 0.001, df = 26.073$).

In the subgroup of male patients ($n = 21$), the authors found a comparable correlation of testosterone and personal distress ($r = 0.545, p < 0.05, n = 21$). The correlation between testosterone and personal distress remained significant when conducting a partial correlation with hepatitis and smoking status as control variables ($r = 0.570, p < 0.05, n = 21, df = 17$).

**Discussion**

The results of this study suggest the presence of specific impairments regarding empathic abilities in opiate-addicted patients. The authors found higher personal distress in opiate-addicted patients in
comparison to controls, consistent with prior research. Moreover, a positive association was found between testosterone and personal distress among the patients’ group, pointing to testosterone as a possible biomarker of empathy impairments in opiate-addicted patients, which has not been investigated before.

In contrast to two previous studies, the authors did not find evidence for impairments in cognitive empathy in opiate-addicted patients. One possible explanation might be the use of a self-report questionnaire in the present study. Self-reported cognitive empathy and empathic abilities assessed with a behavioral paradigm typically correlate only poorly; therefore, some authors conclude that self-report questionnaires are better suited for general clinical research. The current findings correspond to the study results of Tomei and colleagues who likewise found no impairments in self-reported cognitive empathy.

Personal distress can be considered a maladaptive, self-focused affective response to negative emotions in others resulting in physiological over-arousal and behavioral withdrawal. Other studies reported associations of personal distress with neuroticism, poor social functioning, and deficits in self- and emotion-regulation abilities. The present study results indicate that opiate-addicted patients are prone to experience intense negative emotional states in social situations and lack coping skills to regulate these emotions. The abilities of opiate-addicted patients to deal with social situations are important in terms of relapse prevention as pointed out by studies examining the motives for drug use. Thus, the concept of personal distress represents a promising area of research for the treatment of opiate addiction.

In contrast to previous studies typically reporting lower testosterone levels in patients with chronic opiate consumption—but consistent with research suggesting an association between impulsivity and opiate use—as well as testosterone—the authors found higher testosterone levels in the patients’ group compared to controls. The effect of opiates on testosterone levels usually becomes apparent several hours after administration; hence, the current results might reflect a dysregulation of testosterone release due to opiate withdrawal. Similar results have been reported for alcohol-addicted patients. Accordingly, a recent study of the authors’ group (unpublished observations) showed higher testosterone levels in opiate-addicted patients before the administration of diacetylmorphine when compared to testosterone levels several hours later as well as a positive association between the decline of testosterone and craving obtained via self-report. Moreover, testosterone seems to play an important role in opiate withdrawal syndrome in rats, with a positive association between testosterone and withdrawal severity. Therefore, one could argue that high testosterone during early withdrawal might be considered a marker for severe opiate addiction.

It seems likely that opiate-addicted patients characterized by high personal distress are prone to experience craving because they long to reduce negative emotions evoked by social situations and lack adequate coping skills. Indeed, heroin craving was associated with increases in reports of feeling sad or angry in a study with ecological momentary assessment of methadone-maintained cocaine- and heroin-abusing patients. Therefore, high personal distress as a trait might be considered a possible marker for severe opiate addiction as well. This conclusion aligns with the results of previous studies reporting a negative correlation between emotional intelligence and addiction severity. There are studies linking personal distress to deficits in self-regulation which is a multifaceted concept that includes the dimension “resistance to impulsivity.” Impulsivity seems to be associated with addiction severity as well, as it is reported to be a predictor of recovery from drug use and linked to unfavorable addiction treatment outcomes such as difficulties in achieving and maintaining abstinence.

The present study suffers from some limitations which might have biased the results reported here. The authors used a cross-sectional design allowing no causal conclusions to be drawn. A prospective study design assessing testosterone levels at subsequent points during the day, examining a possible decline of testosterone and investigating associations with craving and withdrawal severity would have greatly improved the clarity of the current results. The present study included male, as well as female, opiate-addicted patients and controls, but the number of female participants was small. Further studies with an increased number of female participants are necessary to corroborate the current results regarding female opiate-addicted patients.
The sample consists of severely addicted patients taking part in a structured diacetylmorphine maintenance program designed for a patient population previously considered non-responsive to treatment. 

This can be considered an important strength of the study, as this patient population is seldom examined. On the downside, the current sample contains patients with comorbid psychiatric disorders, other substance addictions and health problems which—even though highly typical for this group and, therefore, increasing ecological validity—might have biased the results of the study. These possible confounding factors should be taken into account when interpreting the data. Some patients were treated with psychopharmacological medication, which might have biased the results as well. Further studies monitoring psychopharmacological medication and controlling for comorbid disorders are necessary.

Despite these limitations, the study results advance a significant line of research regarding specific

Table 1. Sample characteristics of the opiate-addicted patients (n = 27) and healthy controls (n = 31).

<table>
<thead>
<tr>
<th></th>
<th>Patients’ group (n = 27)</th>
<th>Control group (n = 31)</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years</td>
<td>41.67 (±8.814)</td>
<td>40.77 (±8.401)</td>
<td>0.394</td>
<td>56</td>
<td>n.s.</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.101</td>
<td>1</td>
<td>n.s.</td>
</tr>
<tr>
<td>Male</td>
<td>21 (77.8%)</td>
<td>23 (74.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>6 (22.2%)</td>
<td>8 (25.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status:</td>
<td></td>
<td></td>
<td>5.555</td>
<td>3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Single</td>
<td>18 (66.7%)</td>
<td>15 (48.4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>2 (7.4%)</td>
<td>9 (29%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Divorced</td>
<td>6 (22.2%)</td>
<td>7 (22.6%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widowed</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Educational level:</td>
<td></td>
<td></td>
<td>−1.662</td>
<td>56</td>
<td>n.s.</td>
</tr>
<tr>
<td>Main school/no degree</td>
<td>3 (11.1%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Main school with degree</td>
<td>9 (33.3%)</td>
<td>7 (22.6%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle school/no degree</td>
<td>0 (0%)</td>
<td>2 (6.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle school with degree</td>
<td>10 (37%)</td>
<td>14 (45.2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Academic high school/no degree</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Academic high school with degree</td>
<td>4 (14.8%)</td>
<td>8 (25.8%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Substance use disorders (other than opiate addiction):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol addiction (F10.2)</td>
<td>4 (14.8%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol abuse (F10.1)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannabinoid addiction (F12.2)</td>
<td>8 (29.6%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cannabinoid abuse (F12.1)</td>
<td>7 (25.9%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzodiazepine addiction (F13.2)</td>
<td>14 (51.9%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzodiazepine abuse (F13.1)</td>
<td>3 (11.1%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine addiction (F14.2)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cocaine abuse (F14.1)</td>
<td>4 (14.8%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other psychiatric disorders:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic mental disorder (F06.9)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schizophrenia (F20.0)</td>
<td>2 (7.4%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delusional disorder (F22)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bipolar disorder (F31)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recurrent depressive disorder (F33)</td>
<td>4 (14.8%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed anxiety and depressive disorder (F41.2)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTSD (F43.1)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD (F90)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined personality disorder (F61)</td>
<td>12 (44.4%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borderline personality disorder (F60.31)</td>
<td>6 (22.2%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status:</td>
<td></td>
<td></td>
<td>24.035</td>
<td>1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Active smoker</td>
<td>25 (92.6%)</td>
<td>9 (29%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-smoker</td>
<td>2 (7.4%)</td>
<td>22 (71%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic hepatitis C virus infection:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infected</td>
<td>21 (77.8%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-infected</td>
<td>6 (22.2%)</td>
<td>0 (0%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Group comparisons regarding testosterone levels and empathy scores between the group of opiate addicted patients (n = 27) and the healthy control group (n = 31).

<table>
<thead>
<tr>
<th></th>
<th>Patients’ group</th>
<th>Control group</th>
<th>t</th>
<th>df</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (pg/mL)</td>
<td>768.78 ± 497.427</td>
<td>361.81 ± 210.231</td>
<td>3.955</td>
<td>34.006</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Personal distress</td>
<td>11.41 ± 3.165</td>
<td>8.84 ± 3.132</td>
<td>3.100</td>
<td>56</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Empathic concern</td>
<td>10.89 ± 2.667</td>
<td>11.52 ± 2.204</td>
<td>−0.981</td>
<td>56</td>
<td>n.s.</td>
</tr>
<tr>
<td>Perspective taking</td>
<td>14.41 ± 3.092</td>
<td>15.65 ± 2.916</td>
<td>−1.568</td>
<td>56</td>
<td>n.s.</td>
</tr>
</tbody>
</table>
impairments in emotional empathy associated with the possible biomarker testosterone in opiate-addicted patients. Should further research corroborate the hypotheses that high testosterone levels during early withdrawal and high personal distress as a trait are markers for severe opiate addiction, these two markers could help identify severely addicted patients. In this case, the Interpersonal Reactivity Index as well as the assessment of testosterone levels during withdrawal could be used as diagnostic measures to distinguish severely addicted patients from less severely addicted patients.

**ORCID**

Katrin Stange http://orcid.org/0000-0002-2771-9627
Eva Janke http://orcid.org/0000-0002-5671-1990

**References**


Annex II

- Lebenslauf und wissenschaftliche Veröffentlichungen
- Erklärung über die selbstständige Verfassung
- Erklärung zur Verfügbarkeit der Originaldaten
- Einverständniserklärung zur Plagiatsüberprüfung
Erklärung über die selbstständige Verfassung

Hiermit erkläre ich, dass ich die Dissertation „Testosterone and associated biological and psychological factors in alcohol and opiate addiction“ selbstständig verfasst habe. Bei der Anfertigung wurden folgende Hilfen Dritter in Anspruch genommen:

- Korrekturlesen: Thilo Janssen und Kerstin Stange.


Klinik für Psychiatrie, Sozialpsychiatrie und Psychotherapie, Medizinische Hochschule Hannover.

Die Dissertation wurde bisher nicht für eine Prüfung oder Promotion oder für einen ähnlichen Zweck zur Beurteilung eingereicht. Ich versichere, dass ich die vorstehenden Angaben nach bestem Wissen vollständig und der Wahrheit entsprechend gemacht habe.

Hannover, 18. Juli 2017
Katrin Stange
Erklärung zur Verfügbarkeit der Originaldaten

Die Primärdaten beider Studien, die Bestandteil dieser Dissertation sind, werden im Archiv der Klinik für Psychiatrie, Sozialpsychiatrie und Psychotherapie der Medizinischen Hochschule Hannover gelagert.

Hannover, 18. Juli 2017

Katrin Stange

Hannover, 18. Juli 2017
Katrin Stange