Peril or Pleasure
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Summary and general discussion
Introduction

Although effective treatments are available for PTSD, improvement of therapy is urgently needed, as about 30% of patients do not respond to treatment or drop-out of therapy (Bradley et al., 2005; Imel et al., 2013). One way to enhance treatment response may be to combine psychotherapy with pharmacological agents, i.e. medication-enhanced psychotherapy (MEP; Dunlop et al., 2012; Johansen and Krebs, 2009). With pharmacological agents such as oxytocin administration, we may be able to target specific factors that are associated with treatment outcome (Koch et al., 2014; Olff et al., 2010; Quirin et al., 2014). Furthermore, gaining more insight in neurobiological and genetic factors associated with PTSD can potentially open up new avenues for research into treatment targets.

In this PhD thesis, our first aim was to provide an overview of the nature of reward deficits in PTSD by systematically investigating literature on responses to positive stimuli in PTSD patients (part I). Our second aim was to explore if intranasal oxytocin administration can beneficially affect (social) reward functioning and functional connectivity networks in PTSD, as these are neurobiological correlates of previously identified predictors of psychotherapy success (part II). This can inform us on the potential of intranasal oxytocin to boost treatment response in PTSD. Our third aim was to explore (epi)genetic differences between PTSD patients and trauma-exposed controls, which may give more insight in the mechanisms involved in the neurobiology of PTSD and the genetic susceptibility to PTSD (part III). In this final chapter, we present a short overview of our findings, and subsequently integrate and discuss these in light of current literature and potential clinical implications.
Part I: Reward functioning in PTSD

In Chapter 2 we present an overview of the literature on reward functioning in PTSD. Deficits in reward functioning are thought to underlie anhedonia, one of the core symptoms of PTSD. To determine if reward deficits are indeed present in PTSD, we performed a systematic literature review. A total of 29 studies were included, which covered behavioral, subjective and neural responses to positive stimuli (i.e. reward), and investigated reward anticipation and approach (i.e. wanting) and responses to presentation of reward (i.e. liking). Given the PTSD symptoms ‘diminished interest in significant activities’ and ‘inability to experience positive emotions’ (DSM-5; American Psychiatric Association, 2013), we expected reduced reward anticipation and approach responses (i.e. motivational anhedonia) and reduced hedonic responses (i.e. consummatory anhedonia) to presentation of positive stimuli in PTSD patients compared to controls. We were the first to present a systematic overview of the current literature on reward deficits in PTSD. Findings were mixed, but taken together, PTSD was generally associated with reduced anticipatory and approach responses, and reduced hedonic responses. For example, PTSD patients displayed reduced approach behavior, specifically when tasks required more effort. Also, PTSD patients reported reduced positive emotions in response to positive images, suggesting consummatory anhedonia. These reward deficits were observed more often in female than male PTSD samples, and more often in response to social reward stimuli than non-social stimuli. Also on a neural level, altered responses to positive stimuli were observed in the reward pathway and regions involved in social salience processing in the majority of studies. However, studies used a myriad of task-types and paradigms, and results were not wholly consistent. More research is needed to further elucidate the precise nature of reward deficits in PTSD, e.g. separate investigation of reward anticipation and reward presentation. Also, potential sex differences in reward processing in PTSD deserve more attention.

Part II: Oxytocin administration effects on reward and salience neurocircuitry in PTSD

In part II we describe the effects of 40 international units (IU) of intranasal oxytocin administration on neural processing in male and female trauma-exposed police officers with PTSD (n=40, 21 males) and without PTSD (n=40, 20 males). This was investigated in the BOOSTER study, a functional magnetic resonance imaging (fMRI) study, employing a randomized, double-blind, placebo-controlled within-subject design.

In Chapter 3 we reported the effects of intranasal oxytocin on monetary reward and punishment processing in PTSD patients and controls. The monetary incentive delay (MID) task was employed, a well-established fMRI task for eliciting reward pathway responses to monetary reward and punishment anticipation (Knutson). Under placebo, there were no differences in neural responses to reward and punishment anticipation between PTSD patients and controls, contrary to our hypothesis. Anhedonia severity ratings in PTSD
patients were however negatively correlated with ventral striatal responses during reward anticipation, indicating that more severe reward-related symptoms were associated with reduced neural reward sensitivity. Oxytocin administration increased neural responses during monetary reward and punishment anticipation in the striatum, dorsal anterior cingulate (dACC) and anterior insula (AI), in both PTSD patients and controls. These areas are important for initiating approach behavior (putamen), guiding attention towards salient stimuli (AI) and weighing risk and reward options and decision making (dACC). Furthermore, within PTSD patients, oxytocin effects on striatal responses to reward were positively associated with anhedonia, suggesting that oxytocin was most beneficial in patients with high anhedonia symptoms. Through these neural effects, oxytocin administration could potentially increase goal-directed approach behavior and motivational processing, which may improve motivation to initiate and/or continue treatment.

In Chapter 4 we presented how oxytocin administration affected neural responses during a social incentive delay (SID) task (Rademacher et al., 2010; Spreckelmeyer et al., 2009) in PTSD patients and controls. In the SID accurate responses were rewarded with a picture of a happy face, whereas inaccurate responses were punished with an angry face in the feedback phase. Under placebo, PTSD was not associated with different responses to social reward or punishment anticipation, nor to social punishment feedback, compared to controls. However, during social reward feedback, we observed reduced left AI responses in PTSD patients compared to controls. Oxytocin administration normalized these aberrant AI responses in PTSD patients to the level of controls under placebo. Also, oxytocin increased social reward responses in the right putamen in PTSD patients. These effects were specific to PTSD patients, oxytocin reduced AI and putamen responses to reward in controls. Oxytocin administration did not affect responses to punishment anticipation or feedback. Seeing the importance of the AI and putamen in attentional processing, approach behavior and reinforcement learning, we can interpret these findings as indicating that oxytocin administration may enhance sensitivity to social rewarding stimuli, which could potentially benefit social interaction within the setting of psychotherapy treatment.

In Chapter 5, we investigated how oxytocin administration affected neural functional connectivity networks during rest in male and female PTSD patients and controls. We employed resting state scanning, during which participants are asked to relax and let their minds wander. We used independent component analyses (ICA) to identify large-scale intrinsic functional connectivity networks, specifically the default mode network (DMN), salience network (SN) and central executive network (CEN). Under placebo, we observed significantly lower functional connectivity within the SN in female PTSD patients compared to female controls. Oxytocin administration restored SN functional connectivity in female PTSD patients to the level of female controls under placebo. Both male and female PTSD patients also displayed lower mean functional connectivity in the anterior DMN and bilateral CEN relative to controls. Contrary to our hypotheses, these deficits were not alleviated by oxytocin administration. Thus, oxytocin administration
specifically normalized functional connectivity in female PTSD patients in the SN, but not functional connectivity differences in other networks. Deficits in the SN may underlie attentional biases and lack of attentional control observed in PTSD patients (Lanius et al., 2015; Russman Block et al., 2016; Uddin, 2015), which may be alleviated by oxytocin-induced increases in functional connectivity. This in turn may benefit the therapeutic learning process during psychotherapy, for example by facilitating the processing of safety-cues during extinction learning (Cisler et al., 2014).

Taken together, these three neuroimaging studies suggest that oxytocin administration can beneficially affect (social) reward functioning and non-reward related functional connectivity networks in male and female patients with PTSD. Through these mechanisms, intranasal oxytocin may enhance motivation for and sensitivity to social rewarding stimuli and improve attentional control in PTSD patients. This may improve motivation to initiate and/or continue treatment, benefit social interaction within treatment, and facilitate the therapeutic learning process. Thus, oxytocin administration may have potential to boost psychotherapy treatment response in PTSD.

Part III: Genetic and epigenetic associations with PTSD
In part III, we investigated genetic and epigenetic differences between PTSD patients and controls in the sample of the BOOSTER study both in a hypothesis free and a candidate gene driven approach.

Chapter 6 describes an epigenome-wide study of genetic and epigenetic markers based on the Illumina 450k Bead Chip. The Illumina array allowed us to search for CpG-sites (specific locations in the DNA to which methyl-groups can bind, i.e. DNA-methylation) that were differentially methylated between PTSD patients and controls (differentially methylated probes, DMPs, and regions, DMRs). We also analyzed genetic variants (single nucleotide polymorphisms, or SNPs) underlying methylation patterns for some of these CpG-sites, for association with PTSD. We did not observe any single DMPs that were significantly associated with PTSD. However, gene-set enrichment analysis of the top-100 DMPs showed enrichment of three genes involved the dopaminergic neurogenesis pathway. Also, we observed a DMR in PTSD patients compared to controls in the PAX8 gene, which is involved in thyroid and central nervous system development, and has been associated with schizophrenia and sleep disturbances. Furthermore, five CpG-SNPs were significantly associated with PTSD, two of which were located in genes that have previously been implicated in PTSD and other psychiatric disorders: SKA2 and CACNA1C. We were able to replicate the association between PTSD and the CACNA1C CpG-SNP in an independent replication sample. Considering the small sample size, we want to stress that the current findings must be seen as exploratory and interpreted with caution. If replicated in larger samples, these genetic and epigenetic markers may increase our understanding of genetic susceptibility for PTSD, and could potentially serve as biomarkers for early risk-assessment or diagnosis of PTSD.
Chapter 7 describes a candidate gene approach, in which we investigated whether methylation of the oxytocin receptor gene (OXTR) was associated with PTSD in the BOOSTER sample. We observed that PTSD females exhibited increased OXTR methylation compared to female controls, whereas PTSD males did not differ from male controls. Within PTSD females, OXTR methylation was positively associated with anhedonia/emotional numbing symptom severity and negatively associated with amygdala responses to negative faces relative to positive and neutral faces. These findings fit with previously observed associations of OXTR methylation with mood- and anxiety disorders, and amygdala responsivity (Puglia et al., 2015; Reiner et al., 2015; Ziegler et al., 2015). We lacked expression data, however, increased methylation was previously associated with reduced OXTR expression (Kusui et al., 2001). Possibly, reduced OXTR expression may result in reduced sensitivity to positive social cues in the environment, leading PTSD females to interpret neutral and positive expressions as ambivalent or negative (see also (Koch et al., 2016c) and result in (social) anhedonia and emotional numbing symptoms. Although the current findings demand replication, they suggest that OXTR may play a role in anhedonia and emotion numbing in female PTSD patients.

DISCUSSION AND INTEGRATION OF FINDINGS

In the following part of this chapter, we will integrate and discuss the main findings of the studies described above, in relation to other findings from our group and current literature. Clinical implications of our findings are reviewed, as well as strengths and limitations of our work, and recommendations for future research.

Neural reward processing in PTSD

In our systematic review on reward functioning in PTSD (chapter 2), we described several neuroimaging studies investigating neural responses to positive (social) stimuli in relation to PTSD. Effects were inconsistent between studies, likely reflecting differences in methodology and stimuli used. Yet overall, literature suggests hyporesponsiveness in brain areas important in reward and salience processing, hyperresponsiveness in the amygdala and AI, and possible hyporesponsiveness in the striatum in PTSD in response to presentation of positive stimuli, potentially reflecting reduced hedonic responses. Neural differences were observed particularly in response to social reward presentation in PTSD patients. However, the number of available neuroimaging studies was limited, particularly concerning reward anticipation. We can now add our results under placebo from the BOOSTER study to these findings.

Reward anticipation and presentation

Our BOOSTER findings suggest that reward anticipation is not affected in PTSD: PTSD patients did not show different neural responses compared to trauma-exposed controls during anticipation of monetary or social reward and punishment (chapter 3, 4). Possibly,
PTSD-related neural deficits in the reward pathway may manifest only at higher levels of effort, as was observed in previous behavioral studies on reward anticipation (chapter 2). Alternatively, neural deficits may manifest only in patients with high levels of anhedonic symptom severity. Fitting with the latter, in the BOOSTER study symptom severity of ‘diminished interest in significant activities’ was negatively correlated with ventral striatal responses during monetary reward anticipation in PTSD patients (chapter 4), similar to previous observations during reward presentation (Elman et al., 2009; Felmingham et al., 2014). Larger future studies should further investigate possible anticipatory deficits in PTSD in relation to anhedonia. The current findings described in chapter 3 and 4 do not support the notion of neural hyporesponsivity during reward (or punishment) anticipation as a general deficit in PTSD, but suggest deficits are associated with anhedonic symptoms.

In response to presentation of social reward (i.e. happy faces), PTSD patients demonstrated AI hyporesponsivity compared to trauma-exposed controls (chapter 4). This fits with the notion that PTSD patients may show reduced sensitivity to presentation of specifically social stimuli (chapter 2). We did not observe group differences in response to monetary reward presentation in the current sample (unpublished data), further corroborating that deficits may be specific to social reward presentation. Considering the role of the AI in salience detection and attention, a hyporesponsive AI could result in reduced attention towards positive social cues in the environment, which may explain social withdrawal and social anhedonia symptoms in PTSD.

**Sex differences**

In our systematic review, we observed hyporesponsiveness to reward presentation more often in female than male samples (chapter 2). The BOOSTER study is one of the first reward-focused studies to include females from a non-civilian population with high levels of work-related trauma, i.e. police officers, and to directly compare male and females. Interestingly, no sex differences were observed in our sample in response to monetary and social reward (chapter 3, 4). Though this may be specific to non-civilian samples, it does not support the notion that females with PTSD exhibit stronger reward deficits. The suggested sex-differences in our systematic review were strongly confounded by population and trauma type (i.e. male veteran / combat-related trauma vs. female civilian samples / sexual /childhood trauma). Possibly, previously reported reward deficits are (partly) due to trauma-exposure, considering that a number of all female studies included women with sexual and/or childhood trauma (Frewen et al., 2012b, 2010; Guyer et al., 2006; Steuwe et al., 2015), which is generally associated with more severe PTSD and more severe deficits in overall functioning. Furthermore, (male) veteran samples mostly control for effects of trauma-exposure by including combat-exposed controls, whereas civilian samples more often include non-trauma-exposed control groups, potentially leading to larger (trauma-confounded) effect sizes in civilian samples. Taken together, the current findings under placebo do not support the presence of sex differences in neural responses to monetary and social reward in PTSD.
Responsivity to positive and negative stimuli

Stein and Paulus previously suggested that there may be an imbalance between neural responses to presentation of positive and negative stimuli in PTSD, with an increased sensitivity to negative stimuli and a decreased sensitivity to positive stimuli (Stein and Paulus, 2009). However, while PTSD patients rated the angry faces as significantly more punishing compared to controls, we did not observe differences in neural responses to social punishment presentation in PTSD patients relative to controls. This result is at variance with meta-analytic reports of altered neural responses to non-trauma-related negative stimuli (Hayes et al., 2012a). Also, within the BOOSTER study we observed a valence-dependent imbalance in neural responses to an emotional face matching task; controls exhibited increased amygdala responses to angry and fearful faces relative to happy and neutral faces, whereas PTSD patients did not differentiate between positive and negative facial expressions (Koch et al., 2016c). Most of the previous studies compared neural responses to positive relative to negative stimuli (instead of neutral stimuli), which may be a stronger comparison to detect valence-dependent effects, but which also complicates conclusions on whether differences are driven by reward and/or punishment-related deficits. This issue can hopefully be elucidated in future studies by specifically comparing responses to positive and negative stimuli to neutral stimuli.

Taken together, the findings described in this PhD thesis suggest that generally PTSD patient are not hyporesponsive to monetary reward anticipation. This may still be the case in patients with high anhedonic symptom severity, however. The BOOSTER study findings do corroborate the notion that PTSD patients may be hyporesponsive to social reward presentation, relative to trauma-exposed controls. We did not find any evidence for increased neural sensitivity to anticipation or presentation of negative stimuli, nor for sex differences in reward or punishment sensitivity.

Oxytocin effects

Oxytocin effects on reward & salience

In part II of this PhD thesis, we described that oxytocin administration increased putamen, AI and dACC reactivity during monetary reward and punishment anticipation in male and female PTSD patients and controls (chapter 3). Furthermore, oxytocin increased putamen and AI responses to social reward presentation in male and female patients with PTSD, but not in controls (chapter 4). Lastly, oxytocin administration increased functional connectivity within the salience network, largely consisting of the AI and dACC, in female PTSD patients during rest, but not in controls and male patients, or in other networks (chapter 5). This fits with our hypothesis that oxytocin administration can enhance reward and salience processing in PTSD patients. Yet, we could not corroborate these neural findings with behavioral effects of oxytocin administration specifically on reward functioning, as the used paradigms were not designed to measure behavioral effects. PTSD patients did report reduced levels of anxiety and nervousness after oxytocin administration compared to placebo, which may reflect a reduced sensitivity to
negative stimuli or reduced salience processing of negative stimuli. Based on previous behavioral correlates of increased reward and salience network reactivity (Groppe et al., 2013; Kohls et al., 2013; Mickey et al., 2016; Scheele et al., 2014a, 2013; Striepens et al., 2014; Uddin, 2015), we assume that the oxytocin effects on neural reactivity likely imply enhanced behavioral and subjective reward and salience processing. Oxytocin-induced enhanced putamen, AI and dACC processing of monetary reward and loss anticipation may facilitate approach behavior and allow maximization of reward (chapter 3). Oxytocin-induced enhanced putamen and AI responses to social reward – but not social punishment – presentation, suggests enhanced (positive) subjective salience ratings and a shift in attention specifically towards positive social cues in the environment (chapter 4). This interpretation is supported by the finding of oxytocin-induced enhanced salience network connectivity (chapter 5). However, future research combining neural and behavioral measures is needed to confirm these assumptions.

**Oxytocin theories**

Although neuroimaging studies have reported mostly prosocial and anxiolytic effects of intranasal oxytocin, there are also numerous accounts of anti-social and anxiogenic effects of oxytocin administration (e.g. Bartz et al., 2011a; Domes et al., 2010; Eckstein et al., 2014b; Grillon et al., 2013; Shamay-Tsoory et al., 2009). Several hypotheses have been postulated to interpret these apparently contradicting effects of oxytocin administration (see e.g. Bartz et al., 2011b; Olff et al., 2013; Shamay-Tsoory and Abu-Akel, 2015). The oxytocin effects in PTSD patients in the BOOSTER study seem to support the prosocial, approach-related (chapter 3, 4, 5), and anxiolytic effects of oxytocin (Koch et al., 2016b, 2016c). Our findings in PTSD patients fit with the social salience theory, posing that oxytocin enhances the impact of salient ‘safety’ signals, such as happy faces in the SID task, when individuals consider their environment as safe or positive (Olff et al., 2013; Shamay-Tsoory and Abu-Akel, 2015). This in turn may attenuate stress and anxiety responses such as amygdala reactivity and subjective anxiety and nervousness ratings, as also observed by our group (Koch et al., 2016b, 2016c). Our findings also fit with the general approach-theory, suggesting that oxytocin enhances the biological readiness for approach-behavior, also if stimuli are non-social (Harari-Dahan and Bernstein, 2014), such as the monetary reward and punishment anticipation cues.

However, when taking a closer look at the observed oxytocin effects in the control group, a more complex picture emerges. Whereas oxytocin administration increased reward pathway responses during monetary reward anticipation similarly in PTSD patients and controls, oxytocin administration decreased putamen and AI responses to social reward presentation in controls, in opposition to the oxytocin effects in PTSD patients (chapter 4). Similarly, in response to another task intranasal oxytocin increased amygdala responses to emotional faces in controls, which is suggestive of anxiogenic effects, and contrasts with reduced amygdala responses in PTSD patients (Koch et al., 2016c). During rest and an emotional distraction task, oxytocin administration had specific effects on functional
connectivity and reactivity of the amygdala and the salience network in male and female PTSD patients, whereas no effects were observed in controls (chapter 5; Koch et al., 2016b, in prep.). Thus, in nearly all paradigms included in the BOOSTER study oxytocin administration had null- or even opposing effects on affective and salience processing in healthy controls, relative to PTSD patients.

**Interindividual differences**

How can we reconcile these differences between PTSD patients and controls? Our repeated observations of differential effects between psychiatric patients and controls fit with the notion of interindividual differences in oxytocin effects (Bartz et al., 2011b; Olff et al., 2013). One common denominator of oxytocin effects in PTSD patients in the BOOSTER study, is that nearly all of the oxytocin effects normalized aberrant neural responses to the level of controls under placebo and that hardly any other neural effects of oxytocin administration were observed. This suggests that individual differences in baseline functioning may play a role in explaining the differential effects of oxytocin administration. Our findings are similar to prior accounts in psychiatric patients, in which oxytocin administration dampened aberrant amygdala connectivity and reactivity in anxiety- and affective disorder patients, but did not affect, or even increased, amygdala functioning in healthy controls (Bertsch et al., 2013a; Dodhia et al., 2014; Gorka et al., 2015; Labuschagne et al., 2010). Also, a recent study from our group demonstrated that repeated oxytocin administration immediately after trauma reduced development of PTSD symptoms only in participants with high initial PTSD symptoms (van Zuiden et al., 2016). Several studies in healthy participants have also reported stronger effects of oxytocin administration on social reward functioning in participants with less advantageous functioning at baseline (e.g. Bernaerts et al., 2017; Groppe et al., 2013). According to this view, (beneficial) effects of oxytocin administration may only be present or be more prominent in groups showing suboptimal functioning at baseline, i.e. those who have more to gain with regard to social affective functioning (Weisman and Feldman, 2013). This is in line with our findings that also within the PTSD group, oxytocin administration effects were dependent on baseline anhedonic symptoms (chapter 3) and self-reported levels of anxiety (Koch et al., 2016c).

**Oxytocin-response curves**

On the flip side of the coin, oxytocin effects may diminish, level off or even reverse at ‘normal’/higher levels of functioning, e.g. in healthy controls. This is suggestive of a ceiling effect, or inverted-u-shaped response curve, dependent on baseline functioning (figure 1) (Feng et al., 2014; Rilling et al., 2014; Weisman and Feldman, 2013). This response pattern may in part be dependent on interactions with the dopamine system, which is also thought to depend on individual dopamine levels (e.g. Cools and D’Esposito, 2011). Increased central oxytocin levels enhance dopamine functioning in the striatum and mesocorticolimbic dopamine reward pathway (Dölen et al., 2013; Romero-Fernandez et al., 2013; Young and Wang, 2004), which in turn can increase reinforcing properties
and detection of environmental salience signals (e.g. Bromberg-Martin et al., 2010). Individuals greatly differ in dopamine responsivity to external cues (e.g. striatum and insula), which has been related to individual differences in sensitivity to risk and reward (e.g. Smillie and Wacker, 2014; Treadway et al., 2012b). Thus, interactions between oxytocin and dopamine may potentially explain (part of) the interindividual effects of oxytocin administration in both the social salience and the general approach-avoidance theories (Harari-Dahan and Bernstein, 2014; Shamay-Tsoory and Abu-Akel, 2015; Weisman and Feldman, 2013). Although the number of studies of dopamine function in PTSD is limited, PTSD was previously related to increased dopamine transporter (DAT) availability in the striatum compared to trauma-exposed controls (Hoexter et al., 2012), suggesting reduced synaptic dopamine activity, as well as reduced dopamine transmission in response to symptom provocation in PTSD patients (Geracioti et al., 2013). Also in the current sample, we observed enrichment of differential methylation in dopamine-related genes between PTSD patients and controls (chapter 6). Within the BOOSTER sample, we further observed reduced endogenous oxytocin levels in PTSD males compared to male controls (Frijling et al., 2015), and increased OXTR methylation in female PTSD patients compared to female controls (chapter 7). These findings may reflect baseline differences in the endogenous oxytocin system, that may underlie the observed baseline differences in neural responsivity between patients and controls (e.g. chapter 4, 5; Koch et al., 2016b, 2016c), as well as (part of) the differential effects of oxytocin on social reward function in PTSD patients and controls.

Yet, the lack of beneficial oxytocin effects in control participants in the BOOSTER study remains difficult to reconcile with prosocial and anxiolytic findings in healthy subjects reported in non-psychiatric literature. Also, the inverted-U-shaped response curve is at odds with a small number of studies reporting oxytocin’s strongest effects in participants with highest levels of social- functioning at baseline, such as high empathy scores (e.g. Perry et al., 2015) and low autistic traits (Scheele et al., 2014a), as well as in participants with supportive relative to adverse childhood experiences (Bakermans-Kranenburg et al., 2012; Bartz et al., 2010; Bhandari et al., 2014). A possible explanation is that we administered a relatively high dosage of oxytocin in the BOOSTER study (40 IU) compared to most studies in healthy individuals (mostly 24IU), which may have caused a relative ‘overdosing’ or flattening of oxytocin effects. This fits with findings of a dose-response study of oxytocin administration in healthy men, in whom 24 IU of intranasal oxytocin dampened stress-responses, but 48 IU had no effect (Cardoso et al., 2014). Furthermore, we must keep in mind that controls in most psychiatric studies form a selective group devoid of lifetime psychiatric symptoms, whereas in most population-based studies participants with mild psychiatric symptoms may have been included. In the BOOSTER study the controls even form a very resilient group, showing no psychopathology despite high levels of trauma-exposure. This is a potential alternative explanation of differences in oxytocin-effects compared to other groups of healthy individuals.
Figure 1. Hypothesized inverted-U-shaped response curve of oxytocin administration on reward functioning. At baseline (placebo) PTSD patients (indicated with the red dot) are hypothesized to have relatively low oxytocin signaling, related to poorer reward functioning, and are therefore located on the left side of the U-curve. Healthy controls (indicated with the green dot) are hypothesized to have normal oxytocin signaling, related to normal/good reward functioning, and are therefore located near the peak of the U-curve. Intranasal oxytocin administration is thought to cause a temporary increase in oxytocin signaling, indicated with arrows to the right (low dosage with solid line, high dosage with dotted line). In PTSD patients, both low and high dosages are hypothesized to increase reward functioning to higher levels. In healthy controls, who already show adequate levels of oxytocin signaling, a low dosage may have no effect on reward functioning, whereas high dosages may even have a negative effect.
**Sex differences**

Within PTSD patients, we also observed significant sex-dependent effects of intranasal oxytocin on resting state functional connectivity and neural responsivity during an emotional distraction task (chapter 5; Koch et al., 2016b, in prep.). Several previous studies have reported sex-differences in oxytocin effects in healthy subjects, though direction of effects is mixed (Ditzen et al., 2013; Domes et al., 2010, 2007; Feng et al., 2014; Gao et al., 2016; Hurlemann et al., 2010; Lischke et al., 2012; Rilling et al., 2014; Scheele et al., 2014b). Together, these findings suggest that oxytocin administration effects are not simply anxiolytic in males and anxiogenic in females, as has been proposed before (Domes et al., 2010; Wigton et al., 2015). Again, differences in baseline characteristics indirectly associated with participant’s sex may present a viable explanation, considering that all sex-differences in oxytocin effects in the BOOSTER study were paralleled by sex-differences in neural functioning under placebo (chapter 5; Koch et al., 2016b, in prep.). Also, the two studies investigating the endogenous oxytocin system in the current sample observed sex-dependent differences, i.e. reduced oxytocin levels in male but not female PTSD patient compared to controls (Frijling et al., 2015), and increased **OXTR** methylation in female but not male PTSD patients compared to controls (chapter 7). Diverging neural and oxytocin profiles between male and female PTSD patients under placebo fit with suggested sex differences in the underlying neurobiology of PTSD, such as increased neural responses to stress in women compared to men (Maeng and Milad, 2015). Taken together, differences in baseline functioning may be a general factor explaining the moderation by both sex- and PTSD status on intranasal oxytocin effects.

**Genetics and epigenetics**

Genetic and epigenetic factors may also influence individual differences in intranasal oxytocin responses. Variants within the **OXTR** gene have been observed to moderate effects of intranasal oxytocin (Feng et al., 2015; Marsh et al., 2012; Montag et al., 2013). Within the current sample, **OXTR** methylation was associated with PTSD, PTSD symptoms and amygdala reactivity (chapter 7). Also, **CACNA1C** methylation, which was associated with PTSD status in the current and in an independent replication sample (chapter 6), has been associated with amygdala reactivity (Sumner et al., 2015). Thus, genetic and epigenetic factors may affect baseline oxytocin and amygdala functioning, thereby moderating effects of intranasal oxytocin on neural functioning. Future research may investigate the moderating effects of (epi) genetic factors on oxytocin administration.
CLINICAL IMPLICATIONS

Reward and salience deficits in PTSD

The research presented in this PhD thesis suggests that PTSD patients may be affected by reduced salience and valence attribution to positive social stimuli, and reduced attention towards positive social stimuli. This is worth keeping in mind in clinical practice, as (social) anhedonic symptoms may not only affect the patients’ wellbeing, but can also impact psychotherapy effectiveness by reducing perceived social support, motivation for treatment and therapeutic alliance, thus forming an extra barrier on the path to recovery (Charuvastra and Cloitre, 2008). In MDD, psychotherapy is suggested to be more successful when specifically attending to the experience of positive emotions and approach behavior (Dichter et al., 2009; Dunn, 2012). Indeed, a positive activity intervention significantly improved positive affect and psychological wellbeing, while also reducing anxiety and negative affect, up to 6 months after treatment in a combined sample of PTSD patients and patients with other anxiety and mood-disorders (Taylor et al., 2016). A number of evidence-based therapies for PTSD, such as narrative exposure therapy, already attend to positive (social) experiences. Attention of positive (social) life experiences may be specifically beneficial in patients with social deficits and anhedonic symptoms, who may be at risk for reduced psychotherapy response due to reduced perceived social support, ability to form a therapeutic bond and motivation for treatment.

Oxytocin in treatment

In the previous chapters, we described that intranasal oxytocin administration enhanced neural reward pathway responses (chapter 3, 4) and reduced neural reactivity to negative stimuli in PTSD patients (Koch et al., 2016c), both predictors of treatment response in PTSD (Bryant et al., 2008; Falconer et al., 2013; van Rooij et al., 2016). We hypothesize that through these neural effects, oxytocin may enhance sensitivity for positive (social) stimuli. This in turn may benefit perceived social support, the ability to form a therapeutic bond with a therapist, and the motivation to continue treatment (Olff et al., 2010; Quirin et al., 2014), other strong predictors of psychotherapy response (Clarke et al., 2013; Martin et al., 2000; Ormhaug et al., 2015; Schindler et al., 2013; Thrasher et al., 2010). Our findings also suggest that intranasal oxytocin is easy and safe to apply. The next step would be to investigate the effects of intranasal oxytocin as an add-on to psychotherapy on treatment effectiveness. As medication-enhanced psychotherapy, patients would self-administer intranasal oxytocin prior to treatment. By enhancing reward pathway and salience networks responses, oxytocin may shift attentional bias towards positive (social) cues, potentially enhancing the effects of safety signals and social support from the therapist. By dampening amygdala reactivity and improving amygdala-PFC connectivity, intranasal oxytocin can potentially simultaneously reduce excessive fear responses and increase stress-recovery when patients are confronted with traumatic memories in psychotherapy. This may allow PTSD patients to better tolerate the activation of traumatic memories, without completely shutting down all affective responses, creating
a window of tolerance deemed suitable for e.g. exposure therapy. Combined, this may result in better and/or faster psychotherapy treatment response and lower drop-out rates. Seeing that oxytocin effects were dependent on baseline functioning, and strongest in patients displaying the strongest deficits under placebo (Chapter 3; Koch et al., 2016c), intranasal oxytocin may be specifically beneficial in PTSD patients displaying high levels of anhedonic symptoms and/or anxiety. The current findings may not only be relevant to PTSD, but also for other psychiatric disorders involving (social) reward dysfunction/anhedonia and anxiety, such as MDD, schizophrenia, and social anxiety disorder. Transdiagnostic studies are needed to establish the (neurobiological) similarity of these symptoms between patient populations.

Importantly however, based on our current neural findings we can only speculate on the clinical effects of oxytocin administration in PTSD patients. Furthermore, given the moderating role of interindividual factors on effects of oxytocin administration as observed in ours and other studies, we want to emphasize the need for caution in applying intranasal oxytocin in psychiatry until clinical studies can corroborate the efficacy of oxytocin-enhanced psychotherapy. Intranasal oxytocin is still a fickle friend, and until its effects can be reliably predicted, it is not (yet) suited for clinical practice.

Genetics in treatment
In the future, genetics may play a role in the early identification of people at risk for PTSD development, diagnostic subtyping of PTSD, and/or personalized treatment, as is already applied in cancer research. Thus far, psychiatric disorders have proven difficult to capture in terms of genetic risk factors, but larger sample sizes will increase the likelihood of identification of associated genetic variants (Gratten et al., 2014). However, the variance in psychiatric status explained by single genetic variants will likely be low, and large numbers of (rare) genetic variants will likely be implied. This will limit or at least complicate the practical application of personalized medicine. However, genetic studies may help us understand the neurobiology underlying psychiatric disorders, and suggest novel pathways and potential targets for intervention. Epigenetically associated processes may be modifiable and therefore could potentially be targeted directly by interventions. Furthermore, epigenetic markers, such as OXTR DNA-methylation, may provide information concerning endogenous oxytocin functioning, which in turn may predict intranasal oxytocin effects. Thus, (OXTR) DNA-methylation may hold promise in personalized medicine as a potential predictor of (intranasal oxytocin) treatment response. OXTR methylation may also prove interesting for biological subtyping of PTSD, considering its association with emotional numbing symptoms and neural responses to social affective stimuli (chapter 7). We still have a long road ahead, but with the formation of the PTSD working group of the international psychiatric genetics consortium (Logue et al., 2015), and with increasing sample sizes, better phenotyping and advancing methods, exciting times lie ahead in this field.
STRENGTHS, LIMITATIONS AND FUTURE DIRECTIONS

To the best of our knowledge we are the first to investigate effects of oxytocin administration on neural reward functioning in a psychiatric population. Reward functioning and oxytocin administration in PTSD are relatively uncharted areas of research, to which the current studies have added significantly. Also, we have performed one of the first studies reporting on sex-dependent effects of oxytocin administration in a psychiatric population, and of OXTR methylation in PTSD. As observed in previous studies, oxytocin effects in PTSD patients were modified by sex, potentially due to sex differences in underlying neurobiology of PTSD. Other strengths include our homogeneous sample. By including police-officers with and without PTSD, our sample was very homogeneous in terms of trauma-exposure. This allowed us to control for potentially confounding effects of trauma-exposure, which is known to affect neural functioning independent of PTSD symptoms (e.g. Kennis et al., 2015; van Rooij et al., 2014a). We are also one of the first PTSD neuroimaging studies to include both males and females with non-civilian, work-related trauma, granting us a first insight in sex-effects on neural functioning in non-civilian PTSD.

However, these strengths simultaneously constitute limitations; the specific sample of police officers makes comparisons with, and generalizability to other PTSD populations difficult. Also, some of our findings may be related to resilience of the controls, rather than PTSD status of the patient group. Future studies should investigate if our findings can be replicated in other PTSD samples, such as patients with civilian trauma.

We included females using hormonal contraceptives and menopausal females, and did not control for menstrual phase; this allowed us to plan the two scan sessions at short intervals, thereby limiting fluctuations in PTSD symptom severity. However, fluctuations in steroid hormones may have influenced oxytocin effects and (OXTR) methylation (e.g. Caldwell et al., 1994; Scheele et al., 2015). Contraceptive use and menopausal status did not differ between PTSD patients and controls, and were not associated with OXTR methylation. Effects of menstrual phase will likely have evened out between placebo and oxytocin conditions, but this does form a potential confounder that may be investigated in future research.

Another important limitation is the general lack of observed behavioral and subjective effects of oxytocin. We did observe a reduction of self-reported anxiety and nervousness after oxytocin administration relative to placebo in PTSD patients. However, the reward tasks were not designed to measure behavioral differences and subjective reward measures were taken in retrospect, outside the active time-window for oxytocin effects. In future research, it is important to allow better measurement of behavioral and subjective effects of oxytocin, which will support the interpretation of neural effects. Furthermore, we used a saline solution as a placebo, instead of a vehicle solution with similar taste.
and smell, which may have affected the awareness of medication allocation. Subjective awareness of medication allocation was above chance level in the second scan-session in the PTSD group, although smell or taste were not the main reasons reported for correct awareness (chapter 4; Koch et al., 2016b).

A final but important limitation is the small sample size, specifically for our genome-wide (epi)genetic analyses. While we were able to replicate some of our findings in an independent sample, and our results seem to replicate previous observations in PTSD, the current results must be interpreted as exploratory. The BOOSTER study was relatively large for a psychiatric neuroimaging study, but also for our neuroimaging analyses, sample size remains an issue, especially for detecting moderators of oxytocin administration effects. Considering the number of potential moderators (e.g. participant characteristics, task and contextual factors, oxytocin dosage), of which we were only able to test a few, future studies need considerable sample sizes to further disentangle these interactions. Taken together, as for every isolated study, our neuroimaging and genetic findings must be interpreted with caution and replicated is warranted.

Future research may tackle some of the limitations mentioned above and will hopefully be able to elucidate more fundamental issues in the effects of intranasal oxytocin, such as dose–response curves and the effects of repeated administration (effects that may also differ between populations). Further investigation of interindividual factors mediating oxytocin effects is one of the key challenges of oxytocin research in the near future. In the myriad of differential oxytocin effects reported in literature, it is difficult to discern which interindividual characteristics moderate the effects of intranasal oxytocin. Based on the findings described in this PhD thesis, promising moderating factors are psychiatric status, baseline neural (oxytocin) functioning, and sex. Furthermore, our findings encourage replication studies and further investigation of oxytocin administration effects on psychotherapy efficacy. A clinical trial on intranasal oxytocin-enhanced psychotherapy is the next step to test if the potential of oxytocin administration holds true in a clinical setting, with special attention for when and how it should be administered (timing and dosage) and in which patients. Especially in clinical trials, careful monitoring of potential detrimental effects is crucial.
OVERALL CONCLUSION

In this PhD thesis, we have described several studies investigating reward deficits in PTSD patients, the neural effects of oxytocin administration in PTSD patients, and (epi)genetic associations with PTSD. We have provided a first systematic review of reward literature in PTSD, suggesting that deficits in anticipation, approach and hedonic responses to reward are present in PTSD patients and may specifically underlie anhedonic symptoms. Our systematic literature review suggested deficits in specifically social reward functioning in PTSD, which was confirmed in our neuroimaging studies. Next, we demonstrated that as hypothesized, intranasal oxytocin is indeed a promising pharmacological agent to enhance (social) reward and salience processing in patients with PTSD. We suggest that enhanced (social) reward processing may increase psychotherapy success by enhancing sensitivity for subjective social support, therapeutic alliance and motivation for treatment. Increased therapy success is crucial in treatment of PTSD, as still 30% of patients do not respond well to therapy. However, oxytocin effects were strongly moderated by PTSD diagnostic status and sex, likely dependent on differences in baseline neural functioning. Based on these first promising findings that oxytocin administration can enhance neural reward processing and dampen fear processing, the next steps are to investigate the effects of intranasal oxytocin administration on treatment efficacy, and further elucidation of the interindividual factors that may impact (clinical) effects of oxytocin.

Although the scientific community has abandoned its hopes for oxytocin as an all-healing miracle cure, and rightfully so, oxytocin certainly still seems to hold promise in psychiatry. The key will lie in figuring out the where, when and who of its favorable effects. With its contradictory effects, oxytocin may have aroused further scientific interest and fascination, and exciting times are to come when we learn more about the intriguing working mechanisms of oxytocin.