A neuroendocrine perspective on anxiety-related behaviour in humans: focus on testosterone and oxytocin.

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Abstract

Accumulating evidence indicates that the steroid hormone testosterone and the neuropeptide oxytocin may have anxiolytic effects in humans, albeit via different mechanisms. In animals, the anxiolytic properties of testosterone and oxytocin are relatively well-established. In this review, findings of the anxiolytic effects of testosterone and oxytocin in animals are first discussed as well as the possible neurobiological mechanisms by which these hormones exert their effects. Then, a transition to human studies is made and finally the potential of testosterone and oxytocin for the treatment of Social Anxiety Disorder is addressed. From the evidence reviewed here it can be concluded that testosterone promotes active dealing with threat. It enhances attention to threatening social stimuli in an approach-related manner and reduces fear in (socially) challenging situations. Oxytocin on the other hand can have soothing effects, because it reduces background anxiety and acts to re-establish homeostasis in the face of threat. It promotes the processing of social cues, in particular positive social cues. Similar to testosterone, oxytocin enhances social approach behaviour but in an affiliative rather than challenge-orientated manner. Neurobiological pathways involving the amygdala seem implicated in these effects.

Introduction

Over the past decade there has been increasing interest in the roles that steroid hormones and neuropeptides play in the modulation of human social-emotional behaviour. Especially the steroid hormone testosterone (T) and the neuropeptide oxytocin (OT) have received much attention, by the scientific community as well as by the general public. Where T is often associated with aggression and violence, OT is often referred to as the `cuddle-' or `love hormone', suggesting that these hormones have opposite effects on behaviour.

Although these are popularized views, they have of course not come out of nowhere. Bos, Panksepp, Bluthé, & van Honk (2012) have reviewed the effects of OT and T on social-emotional behaviours in animals and humans. They suggest that T initiates approach motivation in the context of desired rewards such as sex, social status or monetary gain. T seems to prepare an individual for action in the face of (social) challenge. A drive which in some situations could lead to aggression.

The terms `cuddle-' and `love hormone' refer to OT's involvement in social bonding. In the context of mother-infant interactions, OT facilitates milk ejection and labour (Gimpl & Fahrenholz, 2001) and also stimulates maternal care behaviour and mother-infant bonding. However, the effects of OT extend beyond mother-infant bonding. Evidence suggests that it can also increase bonding and care between other family members, partners and members of a social group. These effects are likely established through mechanisms such as enhanced social (re)cognition and empathy (Bos et al., 2012). The authors also propose that OT functions primarily in safe environments and that it acts to re-establish homeostasis when homeostasis is disturbed by for example a threat in the environment.

In sum, T and OT seem to play a role in a broad spectrum of social-emotional behaviours ranging from behaviour related to threat and reward, sometimes leading to aggression against conspecifics (T), to behaviour related to the social in-group, such as mother-infant and partner bonding (OT) (Bos et al., 2012). However, an aspect that they seem to have in common is that they both facilitate approach behaviour, particularly in a social context. Approach behaviour can be brought about by a reduction in fear and anxiety, which leads to the hypothesis that T and OT both have anxiolytic actions, especially in social situations.

T and OT have been found to affect neural processing in key areas of emotion regulation such as the amygdala (Bos et al., 2012), making this a plausible hypothesis. Furthermore, animal studies have already provided substantial support for a role of T and OT in the reduction of anxiety-related behaviour (for reviews see: Rotzinger, Lovejoy, & Tan, 2010; Toufexis, Myers, & Davis, 2006). Recently some efforts have been made to translate these findings to humans. However, most reviews regarding effects of T and OT to date have focussed on social cognition and behaviour, only marginally addressing their fear- or anxiety-reducing properties. It is possible that anxiety-reducing properties are in fact essential for T's and OT's modulation of social cognition and behaviour and for that reason they deserve more attention. Therefore, the aim of this review is to summarize the current state of knowledge concerning the modulation of anxiety-related behaviour by T and OT in humans. Since the study of the effects of steroids and peptides on human social-emotional behaviour has only recently begun, findings in animals will first be discussed. Furthermore, this review will address the underlying neurobiological mechanisms by which the anxiolytic effects could occur and the therapeutic potential of T and OT in relation to anxiety.

Anxiety-related behaviour in animals

Testosterone

Possible anxiolytic effects of T have been investigated in a variety of animal species, but mostly in rodents. Pharmacological enhancement of T in rats resulted in increased exploration of the open arms of the elevated plus-maze, although this effect only occurred after 1 week of treatment and not after 2 weeks of treatment (Bitran, Kellogg, & Hilvers, 1993). Effects can thus depend on treatment duration, perhaps due to tolerance. However in the study by Bing et al. (1998) a single dose of T and an 8 week treatment were equally effective in reducing avoidance of punishment during the Vogel conflict test. A single dose of T also reduced anxiety on the elevated plus-maze (Aikey, Nyby, Anmuth, & James, 2002), although T levels had to be much higher than baseline in order to produce this effect. This result suggests that T has to be converted into neurosteroid metabolites first.

Another line of research compared gonadectomized to intact rodents and to gonadectomized rodents with T (or metabolite) replacement. Gonadectomized rats displayed more anxiety-related behaviour on the elevated-plus maze (Frye & Edinger, 2004; Frye & Seliga, 2001), the open field task, the defensive freezing task (Frye & Edinger, 2004), the light-enhanced startle task (Toufexis, Davis, Hammond, & Davis, 2005) and freezing after exposure to a predator's scent (J. A. King, De Oliveira, & Patel, 2005), compared to intact male rats and/or gonadectomized rats with replacement. T treatment can counteract the anxiogenic effects of gonadectomy in young mice as well as hypogonadism associated with aging (Frye, Edinger, & Sumida, 2008). Some behaviours, such as reduced time interacting with a conspecific and defensive burying, were not restored by replacement of T (Frye & Seliga, 2001).

In addition, some forms of anxiety seem to be enhanced by T. In a study by Agis-Balboa, Pibiri, Nelson, & Pinna (2009) long term T treatment in mice increased contextual fear after a fear conditioning paradigm, although mice were subjected to fear conditioning 24 hours after the last T dose. Consistent with this finding, gonadectomized rats displayed little freezing behaviour related to the context of a fear conditioning task (Edinger, Lee, & Frye, 2004; Frye et al., 2008; McDermott, Liu, & Schrader, 2012), which was restored by androgen administration (Edinger et al., 2004; Frye et al.,

2008). This learned behaviour is proposed to be mediated by the hippocampus. The cued fear memory, which is thought to be mediated by the amygdala, was not affected by T manipulations.

In summary, accumulating evidence indicates that T can have anxiolytic effects in a variety of rodent models of anxiety, but can increase context-related fear mediated by learning processes in the hippocampus. The result of T administration can depend on factors such as baseline activity, dose and treatment duration. In addition, the neurobiological effects of pharmacological manipulations could be very different from the organizational effects of gonadectomy early in life, although they sometimes have similar behavioural consequences. Better understanding of the involved neurobiological mechanisms is warranted to elucidate how these factors influence the outcome of T administration.

The anxiolytic properties of T have been confirmed in other animal species. Long term administration of T rendered heifers and ewes less responsive to non-social fear-evoking situations, such as an unfamiliar environment, a novel object or a surprising event (Boissy & Bouissou, 1994; Vandenheede & Bouissou, 1993). More recently, research in nonhuman primates has also investigated T's anti-anxiety effects, which seem to be more restricted to social situations in these animals. Richards et al. (2009) tested the effects of gonadectomy on social behaviour in adolescent male primates. They found that castration affected responses to social stimuli but not to non-social stimuli. More specifically, castrates preferred spending time in the proximity of a familiar conspecific compared to intact monkeys that spent more time in the proximity of a novel conspecific. On the other hand, castrated monkeys failed to avoid threat-related faces of conspecifics. Fear responses to a plastic snake did not differ between groups. Short or long term treatment with T in pharmacologically hypogonadal male rhesus monkeys did not influence fear responses to novel and threatening objects (Lacreuse, Gore, Chang, & Kaplan, 2012; Lacreuse et al., 2010), but seemed to increase vigilance (Lacreuse et al., 2012) and attention to threatening social stimuli (Lacreuse et al., 2010). Although the primate study by H. M. King, Kurdziel, Meyer, & Lacreuse (2012) suggested that enhanced attention to threatening social stimuli may only occur during early exposure.

Thus, it seems that in nonhuman primates T plays an important role in the response to novel and threatening social stimuli. T increases vigilance and attention to threatening social stimuli and promotes the examination of novel conspecifics, that are still ambiguous in terms of threat. However T did not reduce adaptive fear responses to unambiguous social and non-social threat. T might be important for actively and appropriately dealing with threat.

Oxytocin

The anxiolytic effect of OT is relatively well-established in rodent models of anxiety (for a recent review see: Rotzinger, Lovejoy, & Tan, 2010). Agonistic actions of OT or a different OT receptor agonist generally either reduced anxiety-related behaviour or showed no effect. Results with administration of an OT receptor antagonist are less clear (Rotzinger et al., 2010). For example, it did not affect behaviour on the elevated plus-maze (Lukas et al., 2011). Similar to T, the dose and treatment duration as well as the route of administration and test conditions can all affect the outcome. Baseline neuroendocrine status and gender may also play a role. For example, female OT-deficient mice exhibit increased anxiety-related behaviour (Amico, Mantella, Vollmer, & Li, 2004; Mantella, Vollmer, Li, & Amico, 2003) which is relieved by OT administration, but male OT-deficient mice displayed decreased anxiety-related behaviour (Mantella et al., 2003).

More recent findings provide additional support for an anxiolytic effect of OT, especially related to social situations. A single dose of OT reduced fear responses to a predator and increased social preference (Braida et al., 2012; Febo, Shields, Ferris, & King, 2009). After a social defeat rats display social avoidance, which can be reversed by administration of synthetic OT (Lukas et al., 2011).

However, OT also seems to affect more basal anxiety. In a study by Missig, Ayers, Schulkin, & Rosen (2010) subcutaneously administered OT was found to diminish background anxiety in a fear-potentiated startle paradigm in male rats. However learned responses to a fear-conditioned cue or context were unaffected. This is in contrast with T, which did influence learned fear responses to the

context of a fear conditioning paradigm (see findings reviewed above). The finding with OT was replicated in a follow-up study (Ayers, Missig, Schulkin, & Rosen, 2011). However they also compared the findings with intracerebroventricular administration, which did not affect any of the startle measures. This raises the question of whether OT's effects on background anxiety are produced by central or peripheral mechanisms. In the next paragraph possible neurobiological mechanisms will be discussed.

Neurobiological underpinnings

Testosterone

T is primarily synthesized in testes and ovaries of mammals, although small amounts are secreted by the adrenal glands. T plays a key role in the development of male primary and secondary sex characteristics and is the driving force behind the development of sex differences in the brain, which occurs mainly prenatally (Arnold & Gorski, 1984). Although T is the principal male sex hormone, it has important activational effects in the central nervous system in both sexes. In the above reviewed findings it was hinted that T has to be converted into active metabolites in order to produce its effects on anxiety. There are two routes of conversion possible. T can be aromatized to estradiol or it can be reduced by 5α-reductase to dihydrotestosterone (DHT), which subsequently can be reduced to 3α-Diol by 3α-hydroxysteroid dehydrogenase. Both routes have been implicated in T's anxiolytic effects.

Estrogen receptors are expressed in brain areas implicated in social and emotional behaviour including the amygdala and prefrontal cortex of monkeys, rats and humans (Blurton-Jones, Roberts, & Tuszynski, 1999; Montague et al., 2008; Perlman et al., 2005; Perlman, Webster, Kleinman, & Weickert, 2004; Wang et al., 2004) and estradiol has been associated with anti-anxiety behaviour (Frye et al., 2008; Walf & Frye, 2005). Hence, aromatization to estradiol is a possible pathway for T's effects on anxiety. However, rodents in which this pathway is blocked display normal levels of anxiety (Dalla, Antoniou, Papadopoulou-Daifoti, Balthazart, & Bakker, 2005), suggesting that this pathway is not of crucial importance.

Perhaps the other route of conversion is more important for T's anxiolytic actions. The 5α-reduced metabolites DHT and 3α-Diol appear to have similar efficacy to T in reducing anxious behaviour (Aikey et al., 2002; Frye & Lacey, 2001) and there is more evidence suggesting that the end product 3α-Diol is mainly responsible for T's effects (Bitran et al., 1993).

3α-Diol has high affinity for GABAa/ benzodiazepine receptor complexes, whereas T and DHT do not (Frye, van Keuren, & Erskine, 1996; Frye, van Keuren, Rao, & Erskine, 1996; Gee, 1988). In contrast, T and DHT act on androgen receptors (Cunningham, Tindall, & Means, 1979; Verhoeven, Heyns, & De Moor, 1975), while 3α-Diol does not (Roselli, 1991). GABAa agonists like benzodiazepines generally have anxiolytic effects (Dalvi & Rodgers, 1996) and GABAa antagonists blocked the anxiolytic effect of T (but also reduced locomotor behaviour) (Aikey et al., 2002). Thus, agonistic actions of 3α-Diol on GABAa receptors provide for a possible mechanism by which T reduces anxiety. In accordance with this, T increases GABAa receptor sensitivity, likely through agonistic actions of 3α-Diol (Bitran et al., 1993). However this increase in sensitivity of GABAa receptors was diminished after 2 weeks of T administration, as were the anxiolytic effects. This suggests that tolerance or other adaptations might occur after long term exposure to excessive levels of T, perhaps providing an explanation for mood disturbances and irritability observed in long term steroid abusers (Bitran et al., 1993). Agis-Balboa et al. (2009) also propose that T mediated changes in GABAergic neurotransmission in the corticolimbic circuit, especially after chronic treatment, may underlie excessive contextual fear and other mood-related symptoms in T abusers. Thus, as mentioned before, dose and treatment duration are important factors in moderating T's effects on anxiety. However in humans, the prevalence of neuropsychiatric symptoms associated with abuse of

anabolic-androgenic steroids is low, and the symptoms are varied. Anxiety symptoms are in fact among the least reported symptoms (van Amsterdam, Opperhuizen, & Hartgens, 2010).

One of the possible sites of action of androgens is the hippocampus (Collinson et al., 2002; Sar, Lubahn, French, & Wilson, 1990; M. D. Smith, Jones, & Wilson, 2002). The hippocampus seems implicated in the reduction of some anxiety-related behaviours, modulated by T or its 5α-reduced metabolites (Frye & Edinger, 2004). 5α-reduced metabolites also appear to be responsible for the enhancement of the hippocampus-mediated context related freezing in the conditioned fear task, without affecting the amygdala-mediated part of the conditioned fear task (Edinger et al., 2004; Frye et al., 2008). The hippocampus contains androgen receptors (Sar et al., 1990) and GABAa receptors (Collinson et al., 2002) and 3α-Diol enhanced sensitivity of GABAa receptors in the hippocampus (Frye et al., 2008), implying that this might be an important mechanism for some of T's effects. However, 3α-Diol can also affect estrogen-β receptors in the hippocampus (Edinger & Frye, 2007; Pak et al., 2005) and may also affect NMDA receptors or signal transduction pathways (Rhodes & Frye, 2004), indicating that there are many possible pathways that could play a role.

In addition, there are many other brain areas that may be involved in T's effects on anxiety. Androgens can have rewarding effects and mediate anxiety on the elevated plus-maze, through actions in the nucleus accumbens (Frye, Rhodes, Rosellini, & Svare, 2002; Martínez et al., 2002). Additionally, androgens can attenuate light-enhanced startle, possibly via arginine vasopressin in the bed nucleus of the stria terminalis, without influencing fear-potentiated startle mediated by the amygdala (Toufexis et al., 2005). However, T does interact with vasopressin at the level of the amygdala, because it has been demonstrated that vasopressin administration into the amygdala increases aggression through a mechanism involving T (Koolhaas, Van Den Brink, Roozendaal, & Boorsma, 1990). Vasopressin in the bed nucleus of the stria terminalis and other limbic areas is also thought to mediate the regulation of the HPA-axis by T (Viau, Soriano, & Dallman, 2001). T reduces the HPA-axis response to stress (Boissy & Bouissou, 1994; Viau, 2002; Viau & Meaney, 1996; Viau et al., 2001). Since T can directly affect various neurotransmitter or neuromodulator systems and modulate opioid systems, there any many possible mechanisms by which T might influence anxiety (Boissy & Bouissou, 1994).

Oxytocin

OT that is synthesized in magnocellular neurons of the supraoptic nuclei and paraventricular nuclei (PVN) of the hypothalamus is released into the bloodstream, via projections to the posterior pituitary. Peripherally, it can facilitate milk ejection and labour (Gimpl & Fahrenholz, 2001). OT that is synthesized in parvocellular neurons of the PVN has central effects by acting as a neuromodulator. The parvocellular neurons of the PVN project to many brain areas among which the hypothalamus, hippocampus, amygdala, striatum and the brainstem. However, OT's actions are not confined to those areas because it diffuses widely in extracellular fluid (Landgraf & Neumann, 2004) and it can also be released into the cerebral spinal fluid (Veening, de Jong, & Barendregt, 2010). With respect to anxiety, OT pathways in the hypothalamus and hippocampus (Sofroniew, 1983), bed nucleus of the stria terminalis (Ingram & Moos, 1992) and the amygdala (Condes-Lara, Veinante, Rabai, & Freund-Mercier, 1994) are likely involved. OT receptors have also been found in these regions (Bale, Davis, Auger, Dorsa, & Mccarthy, 2001; McCarthy, McDonald, Brooks, & Goldman, 1996). The bed nucleus of the stria terminalis is thought to be implicated in long-lasting anxiety and the central nucleus of the amygdala (ceA) is thought to be more important for the expression of short-term fear (Walker, Toufexis, & Davis, 2003). Indeed, it has been shown that OT receptors in the ceA play an important role in OT's anxiolytic effects (Bale et al., 2001).

Expression of OT receptors in the ceA is restricted to the lateral subdivision (ceL), whereas vasopressin receptors exist in the medial subdivision (ceM) (Huber, Veinante, & Stoop, 2005). Endogenous OT acts on OT receptors in the ceL, where it activates GABAergic interneurons, which subsequently inhibit output from the ceM to the brainstem, causing attenuation of freezing responses (Knobloch et al., 2012; Viviani et al., 2011). The fear-related cardiovascular response was

not affected by ocytocin and it appears to depend on a separate neuronal circuit within the central nucleus of the amygdala (Viviani et al., 2011). On the other hand, activation of vasopressin receptors in the ceM increases output to the brainstem, resulting in opposite effects on anxiety-related behaviours (Viviani & Stoop, 2008). Previously, a similar complementary pattern of expression of OT and vasopressin receptors was found throughout the central extended amygdala (Veinante & Freund-Mercier, 1997), so regions including the bed nucleus of the stria terminalis and parts of the nucleus accumbens may also be implicated in opposing effects in the regulation of stress and anxiety.

Another line of evidence suggests that OT modulates activity within the HPA-axis (Cohen et al., 2010). The authors hypothesize that OT enhances activation of the HPA-axis immediately after exposure to a stressor, which enables quick adaptation of responses. Hereafter, OT facilitates suppression of the HPA-axis through a negative feedback mechanism. This allows for homeostasis to be re-established rapidly. In line with this, OT knockout mice had heightened corticosterone release after a stressor, indicating a dysregulation of the HPA-axis (Amico et al., 2004; Mantella, Vollmer, Rinaman, Li, & Amico, 2004).

The HPA-axis could be a mechanism by which OT influences anxiety peripherally. Peripheral actions of OT may play an important role in anxiety because of OT's inability to cross the blood-brain-barrier (Ayers et al., 2011). Other possible peripheral mechanisms include decreases in heart rate and blood pressure (Gimpl & Fahrenholz, 2001). There is a small portion of OT neurons that project to the posterior pituitary as well as to various regions in the CNS (Gimpl & Fahrenholz, 2001). This may be a mechanism through which peripheral and central OT release occurs in concert to orchestrate anxiety-related behaviour. However, it is not yet clear how exactly peripheral and central release of OT are related and to what extent OT levels measured in blood or in urine correspond to those in the brain (Landgraf & Neumann, 2004).

Anxiety-related behaviour in humans

Testosterone, affective startle modulation and decision-making

In humans, studies investigating the effects of T on anxiety directly are scarce. The first study examining the effect of a single dose of T on a widely established paradigm of fear and anxiety, is the study by Hermans, Putman, Baas, Koppeschaar, & van Honk (2006). These authors examined the effect of T on the fear-potentiated startle reflex in women. A threat of receiving an electric shock reliably potentiated the startle response. It was found that T administration did not affect baseline startle responses, but did diminish fear-potentiated startle. This dissociation between the baseline startle response and fear-potentiated startle has been found before (Baas et al., 2002). It has been speculated that the former reflects background anxiety and the latter cue-specific fear (M. Davis & Whalen, 2001). The authors point out that it is unlikely that the effect seen on fear-potentiated startle is mediated by GABAa/ benzodiazepine receptor complexes, since benzodiazepines mainly affect baseline startle, probably due to their sedative effects (Baas et al., 2002). In contrast, the authors argue that the effect is likely mediated by androgen receptors, which were also found to be responsible for T's effects on defensive burying behaviour (Fernandez-Guasti & Martinez-Mota, 2005)

The effect on cue-specific fear was supported by a second study (Hermans et al., 2007), where T reduced skin conductance and startle modulation in response to negative pictures. However, the latter finding was seen only in a subsample of anxiety-prone participants, perhaps due to a floor effect in the other participants.

A different avenue of research has looked at decision-making, which is influenced by affective signals of punishment and reward. T is thought to enhance the sensitivity to reward and diminish the sensitivity to punishment, which corresponds to reduced anxiety. Indeed, T was found to influence the balance between punishment and reward sensitivity on the IOWA gambling task in a disadvantageous manner (van Honk et al., 2004).

In conclusion, there have been some findings in humans that, in keeping with the animal literature, suggest that T has anxiolytic properties, although some inconsistencies seem to exist: T increased context-related fear and did not influence cue-specific fear in animals (Agis-Balboa et al., 2009; Edinger et al., 2004; Frye et al., 2008; McDermott et al., 2012), but was shown to decrease cue-specific fear in humans (Hermans et al., 2007, 2006). However these experiments seem to tap into different processes. In the animals studies, a fear conditioning paradigm was used and context-related freezing was measured afterwards. The increased freezing response that was measured, is thought to reflect enhanced memory for the context of fear conditioning through mechanisms involving actions of the metabolite 3α-Diol in the hippocampus. In contrast, in the human studies, affect during the tasks was modulated, possibly through mechanisms involving androgen receptors. This is in agreement with studies in rats, in which modulation of affective behaviour is found to coincide with increase in physiological concentrations 1 hour after T administration, while modulation of memory is temporally distinct (Frye & Lacey, 2001).

Since T is thought to play an important role in modulating social behaviour, many studies have been conducted that assess responses to emotional facial expressions. In relation to anxiety, they have examined the influence of T on the attention and neural responses to threat-related facial expressions. These studies are discussed further in this review.

Oxytocin, genetic polymorphisms and the amygdala

In accordance with the animal literature, high OT levels are found to be correlated with low self-reported psychological distress in healthy young adults (Gordon et al., 2008). To further examine the link between the OT system and anxiety some studies have tried to identify genetic vulnerability factors in the OT system that influence anxiety. For example, variations on genes coding for the OT receptor may influence the expression and/or sensitivity of OT receptors, thereby affecting the sensitivity of the system to endogenous OT. Some potentially relevant genetic polymorphisms have been identified so far. In humans a variation on the OT receptor gene (rs2268498) has been implicated in self-reported fear, but only in interaction with a serotonin transporter gene polymorphism (Montag, Fiebach, Kirsch, & Reuter, 2011). Another interaction has been reported between a different OT receptor polymorphism (rs2254298) in adolescent girls and their mothers' history of recurrent major depressive disorder. Girls with the A-allele, whose mothers had recurrent depression, displayed higher scores on self-reported depressive symptoms, physical symptoms of anxiety and social anxiety (Thompson, Parker, Hallmayer, Waugh, & Gotlib, 2011). Although these findings support a role for OT in the modulation of anxiety in humans, it is unclear how the aforementioned genetic variations influence OT system functioning. There is however one study in girls that found that the A-allele of the rs2254298 polymorphism was associated with larger amygdala volumes, providing a potential mechanism (Furman, Chen, & Gotlib, 2011).

There is more evidence for a role of the amygdala in mediating OT's effects on anxiety. Some studies have examined the influence of OT on the amygdala response to threat. For example, one study in healthy males showed that OT reduced amygdala activity. This effect was more pronounced in the condition with threat-related facial expressions than in the condition with threat-related scenes, suggesting a stronger effect on the processing of social information (Kirsch et al., 2005). However, in another study, OT administration in females resulted in heightened amygdala activity in response to both social- and non-social threat (Lischke, Gamer, et al., 2012). These findings suggest that sex differences may exist, although the sample of the latter study was small. Moreover, they suggest that responses to social- and non-social threat may be different. However, since most studies on this subject in humans have used social threat stimuli, this will be the focus of the next paragraph.

Responses to threat-related facial expressions

Testosterone

Most studies investigating effects of T on the processing of facial expressions also check for effects on mood by means of questionnaires. In general, T had no effect on self-reported mood (van Honk & Schutter, 2007; van Honk, Peper, & Schutter, 2005; van Honk et al., 2001; van Wingen et al., 2009), although one study found a positive relation between T levels and self-reported anger and tension 6 hours later (van Honk et al., 1999). Overall, the conscious appraisal of mood seems to be unaffected.

On a behavioural level, high salivary T levels were related to increased preconscious (Wirth & Schultheiss, 2007; van Honk et al., 2000) and increased conscious attention (van Honk et al., 1999) to angry faces during an emotional Stroop task. During a dot-probe task however, high levels of T predicted lower attentional bias to the angry faces (Wirth & Schultheiss, 2007). It might be that T increases approach-related attention on the emotional Stroop task, but reduces the fear-related attentional bias on the dot-probe task.

In the same line, an accelerative cardiac response in response to supraliminally presented angry faces observed after T administration was interpreted as approach-related rather than fear-related (van Honk et al., 2001). Although the approach-related attention to angry faces appears to be enhanced, the conscious recognition of these facial expressions was found to be impaired after T administration (van Honk & Schutter, 2007). Vigilant preconscious attention to fearful faces during an emotional Stroop task was reduced by T administration (van Honk et al., 2005). Taken together, these findings suggest that T increases approach-related attention to angry facial expressions, which signal a dominance challenge. This effect seems to occur on a preconscious level.

Imaging findings may provide an explanation for these effects. In response to angry faces, high T levels were associated with stronger activations in subcortical structures such as the hypothalamus and the amygdala (Derntl et al., 2009; Hermans, Ramsey, & van Honk, 2008). There was also a positive association between T levels and activity in the amygdala in response to fearful faces (Derntl et al., 2009). During an emotional face matching task, T levels were also positively correlated to amygdala reactivity in young and middle-aged women (van Wingen et al., 2009) and in men (Manuck et al., 2010). Middle-aged women on average had lower amygdala reactivity which was restored by T administration (van Wingen et al., 2009). Interestingly, T seems to modulate regulation of the amygdala by the frontal cortex. T levels showed a positive correlation with activity in the superior frontal cortex, but a negative correlation with activity in the orbitofrontal cortex during an emotional face matching task (van Wingen et al., 2009). A follow-up study using the same task showed that T reduced coupling between the orbitofrontal cortex and the amygdala, which may be a sign of reduced regulatory control over the amygdala. On the other hand, coupling of the amygdala with the thalamus was increased (van Wingen, Mattern, Verkes, Buitelaar, & Fernández, 2010).

There are however findings that are not consistent with the above. Stanton, Wirth, Waugh, & Schultheiss (2009) found a negative correlation between T levels and amygdala response to angry faces and a positive correlation with the ventromedial prefrontal cortex response. The amygdala responses and ventromedial prefrontal cortex responses to the angry faces were negatively correlated, which has been found before (Nomura et al., 2004). The authors speculate that participants with high T levels find angry faces less aversive and pay more conscious attention to them, reflected in increased prefrontal cortex activity. A limitation of this study was that these effects were only found in a small sample of men. A study by Volman, Toni, Verhagen, & Roelofs (2011) looked at how endogenous T modulated the control over affect-incongruent approach or avoidance responses, i.e. the avoidance of happy faces and the approach of angry faces. Higher T levels predicted smaller responses of the ventrolateral prefrontal cortex/frontal pole during these responses, but positive coupling of this area with the amygdala. Perhaps this smaller response occurred because for participants with high T levels, the approach of angry faces is more natural, so less affect-incongruent and therefore yields smaller regulatory responses.

In sum, most evidence reviewed above suggests that T enhances approach-related attention to angry faces, while reducing fear-related attention. Furthermore, T seems to promote subcortical processing of these stimuli and diminish cortical processing and regulation, which might render the individual more reactive and prepared for action (see table 1 for a summary of the findings). Consistent with this, a recent study demonstrated that high T was associated with enhanced

biological salience of neutral pictures and increased amygdala activity, although only in men (Ackermann et al., 2012).

It is acknowledged that the findings of increased vigilance for angry faces, increased amygdala reactivity and diminished cortical control over subcortical areas, may also lead to the opposite interpretation, namely that high T can be a risk factor for anxiety. Integration of the literature however, suggests otherwise. It is argued that T increases vigilance for cues signalling threat and reward, by enhancing amygdala reactivity. However sensitivity for reward relative to punishment is increased (van Honk et al., 2004) and an angry face is considered a challenge rather than a threat. It is found that individuals with social anxiety and high cortisol levels, who perceive angry faces as a threat, will direct attention away from these faces (Putman, Hermans, & van Honk, 2004; van Honk et al., 1998). Thus T seems to promote active dealing with threat instead of causing passive fear. Furthermore, the unconsciously detected signals of threat are less likely to reach conscious awareness since cortical-subcortical crosstalk is reduced and explicit recognition of emotional expressions impaired. This might result in less consciously experienced fear and anxious rumination (see also the review about T and social aggression by Terburg, Morgan, & van Honk, 2009). Moreover, the findings in animals and the findings of reduced affective startle modulation after T administration in humans provide further support for the interpretation that T is a protective instead of a risk factor for anxiety.

Oxytocin

As with T, administration of OT had no effect on self-reported mood (Di Simplicio, Massey-Chase, Cowen, & Harmer, 2009; Evans, Shergill, & Averbeck, 2010; Fischer-Shofty, Shamay-Tsoory, Harari, & Levkovitz, 2010; Gamer & Büchel, 2012; Guastella, Carson, Dadds, Mitchell, & Cox, 2009; Guastella, Mitchell, & Mathews, 2008; Lischke, Berger, et al., 2012; Marsh, Yu, Pine, & Blair, 2010; Schulze et al., 2011). This suggests that effects on mood may be more subtle, perhaps only occurring within anxiety-inducing situations. Furthermore, OT may influence emotional processing at a subcortical level not accessible for cognitive evaluation.

However, an influence of OT on emotional processing can be seen in behaviour. Although OT had no effect on response time, accuracy, or gaze toward angry or happy faces in a visual search paradigm (Guastella, Carson, et al., 2009), OT seems to facilitate the recognition of emotional facial expressions (Di Simplicio et al., 2009; Fischer-Shofty et al., 2010; Lischke, Berger, et al., 2012; Schulze et al., 2011), especially happy facial expressions (Di Simplicio et al., 2009; Guastella et al., 2008; Marsh et al., 2010; Schulze et al., 2011). Thus, OT's anxiolytic effect might be secondary to the facilitated processing of positive social stimuli. However, one study suggested that OT also decreased aversion towards angry faces (Evans et al., 2010).

At a biological level, the enhanced processing of emotional expressions was reflected in parasympathetic activity, as OT increased differential heart rate responses to neutral, happy and fearful facial expressions (Gamer & Büchel, 2012). Differential heart rate responses are thought to reflect motivational value of the stimulus (Bradley, Codispoti, Cuthbert, & Lang, 2001). When an individual is better able to differentiate between positive and negative social stimuli, uncertainty and negative interpretation bias regarding a social situation may be reduced, resulting in a subsequent reduction in anxiety.

Since the amygdala is known to be involved in the processing of stimuli with emotional and motivational value, imaging studies have mostly focused on this region. For example the study by (Gamer, Zurowski, & Büchel, 2010) demonstrated diminished responses of lateral and dorsal regions of the anterior amygdala to fearful faces after OT administration but enhanced responses to happy faces. This is consistent with facilitated processing of positive social stimuli, presumably promoting affiliative behaviour.

OT also attenuated the effect of aversive conditioning on negative affective ratings of faces. This effect of OT concurred with a decrease in activity of the amygdala (Petrovic, Kalisch, Singer, & Dolan, 2008). Furthermore, OT reduced activation of the amygdala in response to angry and fearful

faces during an emotional face matching task and reduced coupling of the amygdala to brainstem regions mediating fear behaviour (Kirsch et al., 2005). In support of this, (Domes et al., 2007) also found reduced brainstem activation after OT administration. However, in this study OT decreased amygdala activity for all facial expressions including happy. A possible explanation for this discrepancy is that this study used morphed facial expressions which are more ambiguous. As the amygdala also responds to ambiguity (M. Davis & Whalen, 2001), it could be that OT, by improving the recognition of these facial expression, reduced the ambiguity and thereby the amygdala response for all facial expressions.

In the few studies that have been conducted in females, heightened amygdala activity was shown in response to aversive pictures (Lischke, Gamer, et al., 2012) and fearful faces (Domes et al., 2010) after OT administration. The authors argue that considerable sex differences may exist in the effects of OT, possibly brought about by sex differences in OT receptor affinity mediated by fluctuating levels of steroid hormones. In women, threat perception might be enhanced during certain phases of the menstrual cycle under the influence of steroid hormones. It is important to take these possible sex differences into account.

In conclusion, in most studies amygdala activity in response to emotional facial expressions is reduced and some evidence indicates that related activity in brainstem regions mediating fear behaviour is reduced as well (see table 2 for a summary of the findings). Interestingly, an OT related reduction of activity in the amygdala and other regions involved in fear-processing (midbrain regions) was also seen in association with an increase in trusting behaviour (Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008).

Although threat-related facial expressions are ecologically valid signs of threat, it could be argued that viewing facial stimuli in an experimental setting hardly resembles a real life stressful situation. Therefore, efforts have been made to create experimental settings that entail a real social challenge and that may elicit a considerable amount of stress in some individuals. Unfortunately, these experiments have so far only been carried out with OT administration and not with T administration.

Oxytocin and social stress

A number of OT administration studies have been conducted in which healthy volunteers were subjected to psychosocial stressors. For example, in the experiment by Ditzen et al. (2009) heterosexual couples were instructed to have a couple conflict discussion after they had received either OT or placebo. Couples that had received OT showed more positive communication behaviour during the discussion and had lower cortisol levels after the discussion compared to the placebo group. This suggests that OT can decrease stress by promoting social bonding behaviour.

A more challenging social situation might be the Trier Social Stress Test (TSST). This test involves public speaking and performing a mental arithmetic in front of an audience. Heinrichs, Baumgartner, Kirschbaum, & Ehlert (2003) conducted a study that investigated the effect of OT on this test. They also tested the effect of social support, participants were randomly assigned to bring their best friend to the experiment or to come alone. The results demonstrated an anxiolytic effect of OT and the combination of OT and social support yielded the lowest cortisol levels during stress. In accordance with this anxiolytic effect, Kubzansky, Mendes, Appleton, Block, & Adler (2012) found that participants given OT, compared to placebo, responded to the TSST with a cardiovascular pattern and behaviour that was indicative of a challenge- rather than threat orientation, although in females this challenge orientation was associated with feelings of anger.

In the study by Quirin, Kuhl, & Düsing (2011) a variation on the TSST was used. The cortisol response to public speaking was measured in participants who had either high or low emotional regulation abilities (ERA). Half of the participants received OT before the test while the other half received a placebo. Individuals with low ERA who received OT showed a decreased cortisol response to the stressor in comparison with the low ERA group that received placebo. This suggests that individuals with low emotional regulation abilities could benefit from OT administration.

A similar paradigm was used by D. C. G. de Oliveira, Zuardi, Graeff, Queiroz, & Crippa (2011). OT was linked to reduced subjective anxiety during the pre-test phase and increased sedation during the pre-test phase, anticipation of the speech and delivery of the speech. OT also lowered skin conductance during almost all phases. Other measures, such as cortisol and adrenocorticotropic hormone levels, heart rate and blood pressure, were not significantly affected by OT. The authors suggest that OT is involved in anticipatory anxiety but not in fear of public speaking. This is in agreement with results from animal studies that showed that OT reduced baseline startle responses, reflecting background anxiety, and not fear-potentiated startle (Ayers et al., 2011; Missig et al., 2010). These effects resemble the effects of benzodiazepines (Baas et al., 2002; Zuardi, Cosme, Graeff, & Guimarães, 1993).

OT did not ameliorate affective responses after ostracism during a virtual ball-tossing game (Alvares, Hickie, & Guastella, 2010). The authors argue that the effects of OT are context-dependent and this context of blunt social rejection might be to explicitly averse. It may also be that the outcome measures, consisting of questions which involve explicit cognitive evaluation, are not sensitive enough to detect effects of OT. Biological measures such as cortisol levels, might be more sensitive in this respect.

In summary, the findings indicate that OT can decrease stress by enhancing the effect of social support and positive communication, but can also decrease stress through reduction of anticipatory- or background anxiety similar to the effect of benzodiazepines. However, individual differences play a role, where individuals with low emotional regulation abilities seem to benefit most from OT administration. Possible gender differences should also be taken into account since most studies are conducted in men. Moreover, in women OT seems to be able to induce heightened feelings of anger (Kubzansky et al., 2012) and brain activity thought to be associated with heightened threat perception (Domes et al., 2010; Lischke, Gamer, et al., 2012). Effects of OT also appear to be dependent on the experimental context and outcome measures used. Studies using the same outcome measure are not always consistent. For example, in the study by D. C. G. de Oliveira et al. (2011) cortisol and heart rate measures were unaffected, while they were affected in other studies.

In the review by Bartz, Zaki, Bolger, & Ochsner (2011) the importance is stressed of taking into account individual differences and contextual factors that can moderate the effects of OT administration. Of the findings summarized in their review, 60% showed interactions of OT with moderating factors. These factors should also be borne in mind when considering OT's therapeutic potential. For example, OT is found to improve emotion recognition in individuals with low emotion recognition ability such as autism spectrum disorder patients (Guastella et al., 2010), but not in already high performing participants (Bartz et al., 2010). Moreover, enhancing attention to emotional cues could be detrimental for individuals who are already hypersensitive to these cues and tend to interpret them negatively (Bartz et al., 2011).

Therapeutic potential and future directions

Testosterone

The findings reviewed above suggest OT and T may play a role in anxiety disorders with a social component. To examine the relationship between T and anxiety disorders, salivary T levels were measured in a large sample of participants from The Netherlands Study of Depression and Anxiety (NESDA). Lower T levels were found in patients with depressive disorder, generalized anxiety disorder, social phobia and agoraphobia, although this effect was restricted to females (Giltay et al., 2012). Blood T levels in aging men were not correlated with symptoms of general anxiety, phobic anxiety and panic. However these symptoms did tend to co-occur with a longer genetically determined CAG repeat (CAGn) of the androgen receptor (Schneider et al., 2011). In middle-aged men a longer CAG repeat, yielding relative insensitivity of the androgen receptor, has also been associated with reduced ventral amygdala reactions to threat-related facial expressions (Manuck et al., 2010). This suggest this genetic variation can be a risk factor independent of endogenous T levels.

However, a large population-based study showed that men with T levels in the lowest 10th percentile did have higher anxiety symptom scores, although still at a subclinical level (Berglund, Prytz, Perski, & Svartberg, 2011).

T thus shows promise as a therapeutic agent for men with T deficiency and female patients with anxiety disorders with low concurrent T levels. In women, diminished T levels were seen in generalized anxiety disorder, social phobia and agoraphobia (Giltay et al., 2012), disorders that share symptoms of social withdrawal. Therefore, special interest has arisen for the therapeutic potential in social anxiety disorder (SAD). SAD is a characterized by a persistent fear of one or more social or performance situations in which the person is exposed to unfamiliar people or to possible scrutiny by others (APA, 1994). Patients with SAD typically have exaggerated attention for social threat-related cues (Clark & McManus, 2002; Mogg, Philippot, & Bradley, 2004), which has been linked to hyperactivity in the amygdala (Etkin & Wager, 2007). As discussed above, this exaggerated attention and amygdala activity is thought to be related to avoidance motivation in individuals with social anxiety and high cortisol levels (Putman, Hermans, & van Honk, 2004; van Honk et al., 1998). Since T also enhances attention to angry faces and reactivity of the amygdala, one might be suspect adverse effects when administering T to these individuals. However with T, the increased vigilance is thought to be related to approach motivation. Furthermore T increased sensitivity for reward relative to punishment (van Honk et al., 2004) and reduced cue-specific fear in humans (Hermans et al., 2007, 2006). Therefore, it is predicted that the net effect of T will be that it reduces symptoms of social fear and avoidance in SAD patients by promoting social approach behaviour and active dealing with threat. Studies investigating the effects of T administration in female SAD patients are currently underway.

The different anxiety-related effects of T and how they influence approach behaviour are summarized in the model proposed below (figure 1). Because T influences many mechanisms in the brain and its effects can depend on many factors such as treatment dose and duration, baseline T levels, genetics, gender, personality characteristics and experimental context, its therapeutic potential remains to be further explored. The model in figure 1 could provide a starting point as it might serve to generate new hypotheses. The relations depicted in the model could be tested specifically in SAD patients and it would be interesting to investigate how the aforementioned moderating variables influence these relations.

There is however one concern that deserves to be mentioned. T increased freezing in response to the context of a fear conditioning paradigm in rodents, presumably by affecting memory-related processes in the hippocampus (Agis-Balboa et al., 2009; Edinger et al., 2004; Frye et al., 2008; McDermott et al., 2012). Enhanced memory for context could be detrimental if patients find themselves in explicitly threatening situations. In monkeys, T did not reduce adaptive fear responses to unambiguous social and non-social threat (Lacreuse et al., 2012; Richards et al., 2009). In such a situation, when fear is not reduced by T and the memory for the context enhanced, a fear memory could be the result. Further research is needed to examine how T influences fear memory in humans, for example by studying the effects of T on fear conditioning in healthy humans before studying the effects in patients.

If T is found to be beneficial in SAD patients, the next issue will be feasibility of therapy with T administration. T replacement therapies have not yet been approved for women, but they have been approved for some conditions in men (Corona, Rastrelli, Forti, & Maggi, 2011). In men, T replacement therapy can have antidepressant effects (Zarrouf, Artz, Griffith, Sirbu, & Kommor, 2009). Moreover, T replacement therapy is generally considered safe, as long as serum levels are still within a normal range, although adverse side effects may occur and some concerns exist about the long term effects (Mathur & Braunstein, 2010).

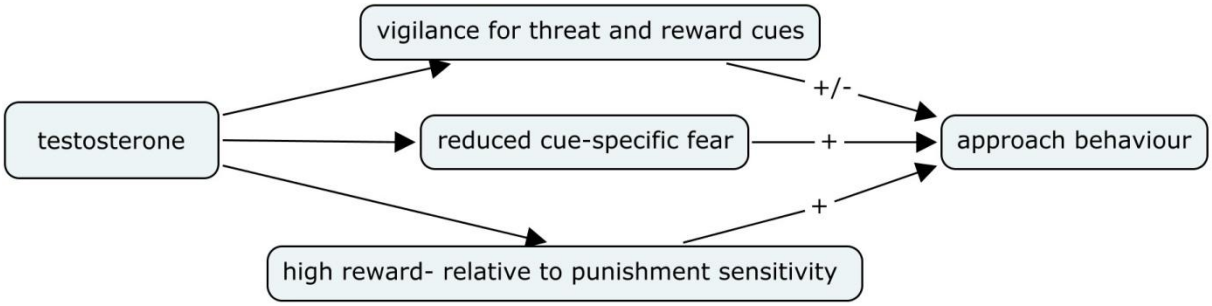

Figure 1. Proposed model of different anxiety-related effects of T and how they influence approach behaviour. The relations depicted in the model could be tested specifically in SAD patients and it would be interesting to investigate how moderating variables, such as context and stable individual differences, influence these relations. Note that increased vigilance for threat may enhance or diminish approach behaviour, but it is expected to enhance approach behaviour because of the concurrent anxiety-reducing effects.

Oxytocin

A similar rationale is proposed for OT. As with T, OT is thought to enhance social approach behaviour, which makes OT particularly relevant for SAD as well. However one study revealed no difference in OT levels between SAD patients and controls (Hoge, Pollack, Kaufman, Zak, & Simon, 2008). In fact, the study showed a positive correlation between OT levels and symptom severity in the patient group, although the sample was small. The authors propose that OT might be elevated to counteract the symptoms of social distress. Indeed, in animals OT is released in response to stress and is thought to subsequently contribute to downregulation of the HPA-axis response (Onaka, 2004). This might indicate that in these patients elevated OT levels are a consequence rather than a cause of stress. In women with depression, peripheral OT release was more variable compared to OT release in healthy controls, which might also be a signal of HPA-axis dysregulation (Cyranowski et al., 2008).

In contrast, in the study by Scantamburlo et al. (2007) symptoms of depression and anxiety were strongly linked to lowered OT levels. In this case lowered OT levels might be a contributing cause to HPA-axis dysfunction. To date, very few studies have examined correlations between endogenous OT levels and symptoms of anxiety and one can only speculate about the causal direction.

Nevertheless, the findings with OT administration reviewed above indicate that OT can relief symptoms by enhancing the recognition of social cues, especially positive social cues. Furthermore, OT reduced amygdala activity in response to emotional facial expressions and some evidence indicates that OT reduced related activity in brainstem regions mediating fear behaviour as well (see table 2). Additionally, studies using a social stress paradigm indicate that OT can decrease stress by enhancing the effect of social support and positive communication (Ditzen et al., 2009; Heinrichs et al., 2003) and can also decrease stress through reduction of anticipatory- or background anxiety similar to the effect of benzodiazepines (D. C. G. de Oliveira et al., 2011). The latter effect was also supported by studies in animals that showed that OT reduced baseline startle responses, reflecting background anxiety (Ayers et al., 2011; Missig et al., 2010).

As mentioned before, some concern was voiced by Bartz et al. (2011), that enhancing the recognition of social cues might be detrimental for individuals who are already hypersensitive to these cues and tend to interpret them negatively. However, since many studies show that OT facilitates processing of positive social cues in particular, the negativity bias in SAD patients may be reduced.

Indeed, this seems to be the case. A small number of clinical studies have been conducted to date, in which OT administration has been investigated in patients with SAD. In the study by Guastella, Howard, Dadds, Mitchell, & Carson (2009) 25 male SAD patients received intranasal OT as an adjunct to exposure therapy involving public speaking. OT did not affect self-reported anxiety during the speech task but improved self appraisal of appearance and performance during the task.

For example, the subjects retrospectively reported making more eye contact and being received positively by group members. Unfortunately, no beneficial effects of OT were seen on the long term. In figure 2 the reported findings are summarized in a model of how OT might facilitate social approach behaviour.

Recent evidence indicates that the beneficial effects that were seen on the short term could have been mediated by the amygdala. In the study by Labuschagne et al. (2010) male patients with Generalized Social Anxiety Disorder (GSAD) exhibited heightened amygdala activity in response to fearful faces, which was dampened by OT. A similar effect was observed in response to sad faces, where hyperactivity in the medial prefrontal cortex and the anterior cingulated cortex in GSAD patients was attenuated by OT (Labuschagne et al., 2011). These findings point to a role for OT in normalizing aberrant brain activity in response to negative (but not necessarily threatening) social cues, which corresponds to the findings in healthy subjects reviewed above.

Although OT administration has promising results, the therapeutic potential seems limited because the of its short lasting effects (about 1.5 hour). Thus, the challenge lies in making the potentially therapeutic effects of OT sustain over a longer period of time, or developing similar compounds that can have longer lasting effects (Bos et al., 2012). As with T, factors should be taken into account that can moderate the effects of OT administration, such stable personality characteristics and context. For example, in women OT seems to be able to induce heightened feelings of anger (Kubzansky et al., 2012) and brain activity thought to be associated with heightened threat perception (Domes et al., 2010; Lischke, Gamer, et al., 2012). Furthermore, some contexts may elicit less positive social behaviours. OT promotes trust and cooperation, but only among in-group members, it increases defensive aggression towards members of the out-group (De Dreu et al., 2010). It has also been shown to increase feelings of jealousy and gloating in humans (Shamay-Tsoory et al., 2009). The model in figure 2 is based on the findings in this review and could serve as a starting point for further questions regarding moderating variables. This model assumes that the enhanced recognition of social cues and the reduction in anxiety are separate processes that occur simultaneously and might influence each other. However, it could also be that reduced anxiety is a prerequisite for the facilitated processing of positive social cues. This is an interesting question that awaits further research.

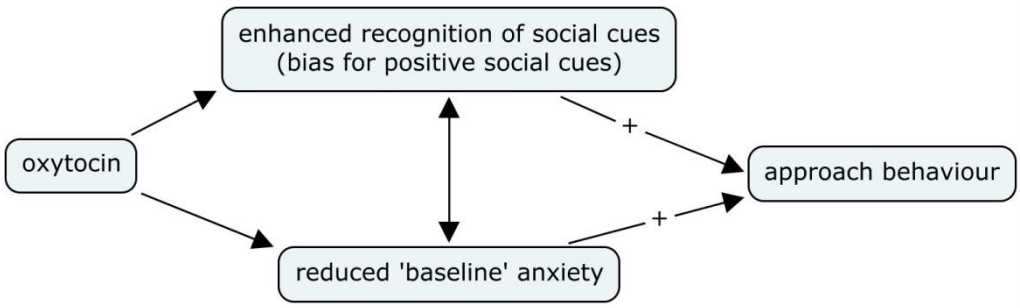

Figure 2. Proposed model of different anxiety-related effects of OT and how they influence approach behaviour. The model is based on the findings in this review and could serve as a starting point to examine further questions regarding moderating variables. This model assumes that the enhanced recognition of social cues and the reduction in anxiety are separate processes that occur simultaneously and might influence each other.

Conclusions

The findings summarized in this review suggest that OT and T indeed have anxiolytic properties. In rodents it is relatively well-established that both OT and T can reduce anxiety-related behaviour, albeit via different mechanisms. There any many possible mechanisms by which T might influence

anxiety. Actions of T's metabolites in the hippocampus seem to play an important role, but regions including the nucleus accumbens, amygdala and the bed nucleus of the stria terminalis are likely involved as well. Moreover, T can inhibit activity of the HPA-axis. For OT there is a strong candidate mechanism which appears to be implicated in its anxiolytic effects. OT can act on OT receptors in a subregion of the amygdala, the ceL, where it activates GABAergic interneurons. These interneurons subsequently inhibit output from the ceM to the brainstem, attenuating fear behaviour. However there are many other regions that may be implicated including the bed nucleus of the stria terminalis, hypothalamus, hippocampus and striatum. Furthermore, OT can modulate activity within the HPA-axis as well. There are many variables that have been found to moderate the anxiolytic effects of T and OT, for example dose, treatment duration and gender. The influences of these variables await further research.

Studies in nonhuman primates suggest that T might be important for actively and appropriately dealing with threat. T did not reduce adaptive fear responses to unambiguous social and non-social threat. However, T promotes the examination of novel conspecifics, that are still ambiguous in terms of threat, and increases vigilance and attention to threatening social stimuli.

Findings in humans seem to be in agreement with this, T enhances approach-related attention to angry faces, while reducing fear-related attention. Furthermore, T seems to promote processing of angry facial expressions in subcortical regions such as the amygdala and diminish cortical processing and regulation, which might render the individual more reactive and prepared for action. Since sensitivity for reward relative to punishment is enhanced and cue-specific fear reduced, the overall result of T administration would be expected to be an increase in approach behaviour (see model in figure 1).

OT on the other hand facilitates the conscious recognition of emotional facial expressions with a bias for positive facial expressions. This seems to co-occur with reduced amygdala activity and some studies suggest that related brainstem activity is reduced as well. It has been argued that a shift occurs from subcortical to cortical processing of social stimuli, opposite to the effects of T. Experiments using social stress suggest that OT can decrease stress by enhancing the effect of social support and positive communication, but also through reduction of anticipatory- or background anxiety similar to the effect of benzodiazepines.

Because both T and OT are thought to enhance social approach behaviour, they show most promise as therapeutic agents in Social Anxiety Disorder. However some concerns were raised. The subcortical vigilance for threat cues caused by T might be expected to be detrimental in these patients. However this vigilance is thought to be approach-related and because of the concurring reduction in fear and increase in reward sensitivity, the net effect is expected to be an increase in approach behaviour. The concerning fact that T can increase fear memory for context in rodents remains an issue that needs to be further explored in humans.

Similar to T, one might expect that enhanced recognition of social cues caused by OT administration might result in adverse effects in SAD patients who are already hypersensitive to social cues and tend to interpret them negatively. However recent findings suggest otherwise since administration in SAD patients resulted in a more positive interpretation of social cues during a socially stressful situation and reduced activity in brain areas that respond to negative social cues such as the amygdala, which corresponds to findings in healthy subjects. Unfortunately, effects of OT are very short-lasting, so the challenge lies in making the beneficial effects of OT sustain over a longer period of time.

In this review a model was proposed of how OT facilitates social approach behaviour (figure 2). This model assumes that the enhanced recognition (with a positivity bias) of social cues and the reduction in anxiety are separate processes that occur simultaneously to facilitate social approach behaviour. However, it could also be that reduced anxiety is a prerequisite for the facilitated processing of positive social cues. This is an interesting question that awaits further research. The models proposed in this review may provide direction for future research to start exploring the moderating effects of context and stable individual differences on the anxiolytic effects of OT and T.

Table 1. Influence of testosterone on the response to threat-related facial expressions

Reference	N	Testosterone measurement/ administration	Key findings
(van Honk et al., 1999)	16 m, 16 f	Salivary T levels	Positive correlation between T levels and self-reported anger and tension, and selective attention to angry faces. Only when saliva samples were obtained 6 hours before testing.
(van Honk et al., 2000)	40 m	Salivary T levels	Post-task cortisol and T levels were increased in participants displaying preconscious selective attention to angry faces.
(van Honk et al., 2001)	16 f	.5 mg T (w)	T induced an accelerative cardiac response to angry faces, which may reflect an inclination to approach.
(van Honk et al., 2005)	16 f	.5 mg T (w)	T reduced the preconscious selective attention to fearful faces. No effect on self-reported mood.
(van Honk & Schutter, 2007)	16 f	.5 mg T (w)	T impaired the recognition of morphed angry faces.
(Wirth & Schultheiss, 2007)	41 f, 29 m (1); 26 f, 26 m (2)	Salivary T levels	(1)Positive correlation between T levels and the reinforcing qualities of subliminal angry faces in a learning task. (2)Positive correlation between morning T levels and preconscious selective attention to angry faces in an emotional Stroop task, but attentional orienting away from subliminal angry faces in a dot probe task.
Imaging studies			
(Derntl et al., 2009)	21 m	Blood T levels	Negative correlation between T levels and reaction times to fearful male faces, not for the other expressions. Positive correlation between T levels and amygdala response to fearful and angry faces, but not for the other expressions.
(Hermans et al., 2008)	12 f	Salivary T levels .5 mg T (w)	High T and low cortisol was associated with stronger activations in subcortical structures in response to angry versus happy faces, such as the amygdala and hypothalamus. T administration resulted in more persistent activations.
(Stanton et al., 2009)	14 f, 10 m	Salivary T levels	Negative correlation between T levels and amygdala response to angry faces. Positive correlation between T levels and ventromedial prefrontal cortex response to angry faces in the passive-viewing task. Amygdala and ventromedial prefrontal cortex responses to angry faces were negatively correlated. These effects were only seen in men.
(Volman et al., 2011)	24 m	Salivary T levels	Higher T levels predicted smaller responses of the ventrolateral prefrontal cortex/frontal pole during affect-incongruent trials, but positive coupling of this area with the amygdala.
(van Wingen et al., 2009)	25 f (middle-aged), 17 f (young)	Blood T levels .9 mg T, nasal (w) (only in middle-aged f)	Positive correlation between T levels and amygdala response across all women. T administration increased the amygdala response of middle-aged women to a level similar to that of young women. After drug administration, the T levels of middle-aged women correlated positively with superior frontal cortex responses and negatively with orbitofrontal cortex responses. (emotional face matching task)
(van Wingen et al., 2010)	25 f (middle-aged)	.9 mg T, nasal (w)	T reduced functional coupling of the amygdala with the orbitofrontal cortex, but enhanced coupling with the thalamus. T also reduced coupling with the contralateral amygdala. (emotional face matching task)
(Manuck et al., 2010)	41 m	Salivary T levels	Positive correlation between T levels and activation of the dorsal amygdala (not affected by AR gene variation). Effect of AR gene variation on activity in the ventral amygdala (not affected by T levels). (emotional face matching task)

T, testosterone; m, male; f, female; w, within subjects design; b, between subjects design.

Table 2. Influence of oxytocin on the response to threat-related facial expressions

Reference	N	Intranasal oxytocin administration	Key findings
(Di Simplicio et al., 2009)	29 m	24 UI OT (b)	OT slowed reaction time to recognize morphed fearful faces and reduced the misclassification of positive facial expressions as negative ones.
(Guastella et al., 2008)	69 m	24 UI OT (b)	OT improved memory for previously seen happy faces compared with angry and neutral faces.
(Guastella, Carson, et al., 2009)	71 m, 33 f	24 UI OT (b)	OT had no effect on response time, accuracy, or gaze toward angry or happy faces in a visual search paradigm.
(Marsh et al., 2010)	29 m, 21 f	24 UI OT (b)	OT improved the recognition of morphed happy facial expressions, irrespective of gender, response biases, or changes in mood. Recognition of other expressions was not affected.
(Evans et al., 2010)	18 m	24 UI OT (w)	OT decreased aversion to select a financially rewarded angry face, without affecting financial reward processing per se and without affecting the selection of happy vs. sad faces.
(Fischer-Shofty et al., 2010)	27 m	24 UI OT (w)	OT enhanced the recognition of fear, but not other emotions.
(Schulze et al., 2011)	56 m	24 UI OT (b)	OT improved detection accuracy for masked emotional stimuli, particularly for happy faces.
(Lischke, Berger, et al., 2012)	47 m	24 UI OT (b)	OT enhanced the recognition of morphed emotional expressions.
(Gamer & Büchel, 2012)	46 m	24 UI OT (b)	OT increased differential heart rate responses to neutral, happy and fearful facial expressions, but had no effect on skin conductance resonses.
Imaging studies			
(Domes et al., 2007)	13 m	24 UI OT (w)	OT reduced right-sided amygdala activity to angry, fearful and happy faces in an implicit recognition paradigm.
(Domes et al., 2010)	16 f	24 UI OT (w)	OT enhanced activity in the left amygdala, the fusiform gyrus and the superior temporal gyrus in response to fearful faces and in the inferior frontal gyrus in response to angry and happy faces.
(Petrovic et al., 2008)	27 m	32 UI OT (b)	OT attenuated the effect of aversive conditioning on negative affective ratings of faces. This effect of OT concurred with attenuation of activity in anterior medial temporal and anterior cingulate cortices. In the amygdala and fusiform gyrus activity this reduction of activity was stronger for faces with direct gaze.
(Kirsch et al., 2005)	15 m	27 UI OT (w)	OT reduced activation of the amygdala in response to angry and fearful faces and reduced coupling of the amygdala to brainstem regions. (emotional face matching task)
(Gamer et al., 2010)	46 m	24 UI OT (b)	OT attenuated activation in lateral and dorsal regions of the anterior amygdala for fearful faces but enhanced activity for happy expressions.

OT, oxytocin; m, male; f, female; w, within subjects design; b, between subjects design.

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