The Role of Incentive Learning and Cognitive Regulation in Sexual Arousal

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Chapter 1

General Introduction

1. General Introduction

In the ancient Greek philosophy as written in Phaedrus dialogue, Plato compares the human mind to a charioteer who is pulled by two horses (Plato, 370-360 BC.). The white horse is obedient, always following the charioteers' instructions, while the black horse, which is representing temptation, is impulsive and irrational, and is always attempting to thwart the journey towards enlightenment and the truth. The value of this chariot allegory is its reflection of human nature, by which Plato laid the foundation of an idea that has fascinated and troubled western scientists and philosophers for centuries (Damasio, 1994; Pessoa, 2008, 2013): *emotions and man's appetites can interfere with reason*. In other words, from the ancient Greek philosophers on, emotions were seen as pernicious influences on judgement and behaviour that therefore need to be controlled by the rational mind.

Although modern psychological science has moved on since antiquity, this illustration of conflict between ratio and emotions still makes sense to us, as it reflects the struggle of temptations and even addictions. Most likely, we all have had experiences with giving in to all kinds of temptations -big or small- we face throughout life, such as with eating that chocolate while on a diet, breaking new years' resolution by drinking that glass of wine, or engaging in sexual activities at moments despite knowing it would be better and wiser not to do so. For most individuals such occasional loss of self-control is relatively harmless, as it will not develop in problematic behaviours. However, it is not hard to see that a structural inability to regulate extremely frequent, strong or suddenly increased sexual urges and sexual arousal can lead to significant personal distress, and impairment in interpersonal, social, and occupational functioning. Resisting temptation exemplifies the competition between brain systems of low-level control and high-level control (Toates, 2014). From the above it is clear that the stimulus itself (e.g. the chocolate, the glass of wine, or a sexual attractive individual) as well as the ability to use self-control and regulate

responses, may contribute to the development of normal healthy behaviours, as well as to the development and persistence of problematic behaviours. For sex, this means that the emotion (i.e. the state of sexual arousal) and cognition both function as control systems that regulate sexual behaviour.

Understanding sexual arousal and behaviour requires a theoretical framework, one that includes influences of both biology and cognition, and which can mesh with evolutionary psychology. Therefore, we will first tap into the constructs of lower-level and higher-level systems of control, where after it will be elucidated that sexual arousal can be seen as an evolutionary preserved emotion. After that we will discuss sexual learning and related lower-level control processes. Subsequently we will discuss higher-level processes, such as cognitive emotion regulation, that are relevant to the understanding of sexual arousal and behaviour. Finally, an outline of this thesis will be given.

1.1. Multilevel Control Systems

Hierarchical models as proposed by LeDoux (2012), Toates (2009, 2014) and Metcalfe and Mischel (1999) suggest there are two levels of emotional stimulus processing. For sex, particular salient stimuli in the environment or in memory may capture our attention automatically (Dekker & Everaerd, 1988; Maner, Rouby & Gonzaga 2008; Toates, 2014) and can act through a lower-level system of control to elicit sexual arousal and to engage appetitive behaviour (Carver, Johnson & Joormann, 2009). This means that this evolutionary old lower-level of control system is reactive to specific stimuli and events that eventually can translate in action readiness (Frijda, 1986). Some stimuli can trigger sexual arousal by rapid automatic unconscious processing (i.e. the so called 'quick and dirty-route'; LeDoux, 2012), and involve activation of subcortical structures (e.g. nucleus accumbens; NAc, ventral striatum, ventral tegmental area; VTA) and temporal cortex (Childress et al., 2008; Oei et al., 2012). For instance, the presence or thought of another attractive individual

may grab our attention and exert a pull (Toates, 2014), and kissing or genital touch may automatically elicit sexual arousal response. Likewise, aversive stimuli or events, such as sexual assault or painful intercourse, can lead to withdrawal from a situation. The lower-level system of control can operate outside awareness, though the endpoint of its processing can engage consciousness. This means that sexual arousal can occur unconscious and individuals are not always able to report their emotional response at the moment it occurs (Childress et al., 2008; Gillath & Canterberry, 2012; Oei et al., 2012). Nevertheless, this emotional response can be visible in their behaviour, in physiological responses or subjective impressions of an-affect laden event. In this way, sexual stimuli can activate bodily responses (and implicit memory) before conscious appraisal (Berridge & Winkielman, 2003). For instance, a sexual stimulus, such as an erotic picture, may trigger a fast and automatic sexual response in women, as measured by increased blood flow in the genitals, in spite of the fact that this does not necessarily correspond to a state of subjective conscious sexual excitement (Laan & Janssen, 2007).

On the lower-level system there are various factors that determine which stimuli and events may serve as salient sexual stimuli and trigger sexual response and approach behaviour. According to incentive motivation models, sexual motivation is the result of the interplay of a sensitive internal sexual system with external motivational stimuli or the mental representation thereof (Ågmo, 1999; Singer & Toates, 1987). With other words, the biological sexual system is sensitive to sexual stimuli is prepared and to processing information relevant for sexual response (Everaerd & Laan, 1995). Neurotransmitters such as dopamine (DA), and hormones such as oestrogens and androgens act on the brain regions involved in sexual arousal and behaviour and sensitize incentive pathways, thereby increasing the likelihood that a given stimulus will trigger approach (Toates, 2014; Wallen & Lovejoy, 1993). Importantly, the strength and the number of sexual incentives vary across individuals, since many not-

intrinsically sexually competent stimuli may acquire the capacity to elicit sexual arousal through basic learning processes such as in classical conditioning. The attractiveness of those stimuli depends on their history of positive or negative behavioural consequences and the resulting meaning that is stored in memory. It is suggested that as a result of aversive conditioned learning, sexual arousal may decrease after negative sexual experiences, such as sexual assault or repeated experiences with painful coitus (Both et al., 2008a; Brauer et al., 2007; van Berlo & Ensink, 2000). Therefore, the role of memories of aversive experiences in problematic sexual desire and arousal is likely to be important.

However, there is only limited empirical research on classical conditioning of sexual arousal in humans, while it is likely to yield important knowledge about mechanisms underlying sexual motivation and related disorders such as problems of low sexual arousal in men and female sexual interest/arousal disorder (Diagnostic and Statistical Manual of Mental Disorders 5, 2013). Female sexual interest/arousal disorder is the most common sexual complaint in women, characterized by a lack of sexual interest, often accompanied by the experience of low sexual arousal, and can cause marked personal distress and marital discord and has a negative impact on quality of life (Mercer et al., 2003; West et al., 2008). Likewise, on the other end of the spectrum, in the aetiology of hyperactive sexual desire such as in hypersexuality or 'sex addiction', and also in paraphilia, basic learning processes are hypothesized to play a pivotal role (Both et al., 2008a,b; Pfaus, Kippin & Centeno, 2001; Pfaus et al., 2013). It is thought that in a similar manner, the automatic physiological sexual response and the positive affect produced by sexual stimulation may become associated with environmental stimuli, and these stimuli thereby become conditioned sexual incentives. Hypersexuality is mostly observed in men, and appears to be associated with paraphilia-related disorders (Kafka, 2007). It encompasses strong sexual desire and repetitive sexual behaviour which is difficult to control, reflected by for instance,

compulsive masturbation, prolonged promiscuity, and dependence on pornography or internet sex (Kafka, 2007). Paraphilias are characterized by intensely arousing, recurrent sexual fantasies, urges and behaviours that are considered deviant with respect to cultural norms and that produce clinically significant distress or impairment in social, occupational or other important areas of psychosocial functioning (e.g., exhibitionism: exposure of genitals to a stranger; voyeurism: observing others' sexual activities; fetishism: use of inert objects) (Kafka, 1993, 1994, 2007). Insight in the underlying mechanisms of sexual motivation is essential to understand disorders in sexual desire and such understanding may be helpful in the development of new and effective psychological and pharmacological interventions, since empirically validated treatments are lacking for female sexual interest/arousal disorder as well as hypersexuality and related disorders (Binik & Hall, 2014; Kafka, 2007; Ter Kuile. Both & van Lankveld, 2010).

Next to the attractiveness of sexual stimuli, cognitive processing is also thought to play an important role in sexual motivation. Notwithstanding the importance of sexual incentives, individuals are not simply driven by external sexual incentives or the cues associated with them. Cognitive processes like conscious awareness, goals and social restrictions can influence this process (Toates, 2014). Further insights in human sexual motivation and behaviour require looking more closely at the pathway of information between stimulus and response and considering how these processes are embedded within other (higher-level) processes and higher order brain structures such as frontal regions, including the prefrontal cortex (PFC) known to be involved in emotional processing and regulation (Spiering, Everaerd, & Janssen, 2003; Janssen et al., 2000; Toates, 2009). Higher-level processes such as reasoning are very relevant to action control, especially during the regulation of emotions. Humans have the ability to process stimuli and situations in a deliberate, controlled and often conscious way. In this way stimulus meaning can be

determined and action predisposed (Frijda, 1986; LeDoux, 2012). Moreover, this cognitive ability allows us to influence and alter emotions by using thoughts. This can be done by reinterpreting the significance of an event or stimulus, or to set the attentional focus on less arousing aspects of a stimulus or situation (Everaerd, 1989; Van Lankveld, Van den Hout & Schouten, 2004). In this way, cognition can be tuned in the service of generating more adaptive emotional reactions. Research on the patterns of brain activation in the cognitive regulation of sexual arousal, suggest that emotional self-regulation depends on a neural circuit in which prefrontal cortical areas mediate the cognitive modulation of emotional responses generated at a subcortical level (Beauregard, 2007). This means that these prefrontal areas are involved in metacognitive and executive top-down processes. These 'higher' cognitive processes can be described as *thinking* and *reasoning* (Evans, 2008).

To conclude, both lower- and higher-level control processes are relevant in the understanding of sexual arousal and motivation, and related disorders. Because much sexual behaviour is thought to be acquired through learning, and given the specific hypothesis that classical conditioning plays an etiological role in the development of sexual dysfunction (Toates, 2009), research in the area of classical conditioning of human sexual arousal is warranted. The next section will discuss the constructs of sexual arousal, sexual learning and related phenomena, and will look subsequently into the unanswered but highly relevant research questions within the field of human sexual incentive learning that relate to these constructs. After that, in a similar manner we will tap into the higher-level processes of control, including how the mental challenge posed by stress may decrease the weight of high-level control and increase that exert at a lower level (Heatherton & Wagner, 2011; Toates, 2014).

1.2. Sexual Arousal

From an evolutionary perspective, emotions represent biologically based modes of adaption to changing environmental demands, which have emerged in the course of evolution on account of their capacity to adequately coordinate the various response systems that characterize emotion's multicomponential nature (LeDoux 2000, 2012; Öhman & Mineka 2001). Sexual arousal can be seen as an evolutionary preserved emotion (Everaerd, 1989; Frijda & Sundararajan, 2007; Janssen, Everaerd, Spiering, & Janssen, 2000). According to Lang (1971, 1995) emotions can be seen as action dispositions, states of vigilant readiness that vary widely in (1) reported subjective apprehension or verbal responses, (2) physiological arousal, and (3) approach/avoidance behaviour. Yet, as already mentioned, these response systems may diverge, illustrated by the observation that a woman can show physical signs of sexual arousal, while no subjective feelings of sexual desire or arousal are reported (Laan & Janssen, 2007; Toates, 2009, 2014). Experienced sexual arousal is founded on the activation of a neural circuit that evolved in the mammalian brain to ensure the survival of the species (LeDoux, 2000, 2012). Sexual arousal is characterized by specific bodily reactions, like enhanced genital blood flow, by preparation of behavioural action, and by the experience of feelings of lust, excitement, and sexual desire, and can eventually result in overt sexual behaviour such as approach and consumption (Both et al., 2005; Dekker & Everaerd, 1989; Lang, 1971). Emotions, including sexual arousal are driven by two opponent motivational systems: appetitive and aversive subcortical circuits that mediate reactions to primary reinforcers (Lang, 1995). The neural circuit of sexual arousal reacts to sexually relevant appetitive environmental and memorial cues, mediating appetitive reflexes that tune sensory systems and mobilize the organism for action (Dekker & Everaerd, 1989; Everaerd, 1989; Georgiadis & Kringelbach, 2012; LeDoux, 2012). In this way the 'sexual-system' helps organisms to detect and approach -through perception, memory and behaviour- those situations

and stimuli that can be sexual rewarding, in order to reproduce. Additionally, emotional responses like sexual response can facilitate rapid action and decision making and enhance our recollection of important events (Phelps & LeDoux, 2005). Conversely, organisms must also identify, learn about, and respond appropriately to cues in the environment which signal threats to survival, or threats to successful reproduction. This means that the motivational systems – appetitive and aversive- are competing and are thought to be mutually inhibitory (Barberini et al., 2012; Nasser & McNally, 2012). The appetitive system mediates approach and rewards, whereas the aversive motivational system mediates avoidance and fear, with recruitment of these systems controlled via associative learning processes that depend on dopaminergic transmission in the brain.

1.3. Sexual Learning

Sexual response can be incited by specific triggers, and encompasses unlearned responses to stimuli with intrinsic sexual emotional value, or learned responses to stimuli with acquired sexual emotional significance. Some stimuli are innately competent in evoking sexual responses automatically (i.e. without deliberate control). Shiffrin and Schneider (1977, p. 155–156) defined an automatic process as *"a sequence of nodes that nearly always becomes active in response to a particular input configuration*". For instance, some sexual stimuli, like for instance kissing or genital touch (the so-called unconditioned stimulus; US) can elicit a range of automatic sexual responses (the so-called unconditioned response) like enhanced genital blood flow, and the experience of feelings of lust and sexual excitement (Dekker & Everaerd, 1989). However, an initial neutral stimulus, such as a neutral picture or a neutral odour can acquire sexual emotional significance through pairing with a sexual rewarding stimulus (US), such as genital stimulation or orgasm, and become a so-called conditioned stimulus (CS) (Pfaus, Kippin & Centeno, 2001). After only a few CS-US pairings, the

presentation of the CS alone is capable of eliciting 'automatically' sexual response (this is now called the conditioned response; CR) (Pavlov, 1927). Classical (or Pavlovian) conditioning separates itself from instrumental (or operant) conditioning, in which the association between a response and its reinforcing or punishing consequences are learned (Skinner, 1937). Sexual learning is necessary to ensure the survival of the species, as it allows an organism to use cues in the environment in order to predict upcoming sexual aversive or rewarding events and is therefore considered of adaptive value. Learning about sexual cues may encompass learning of positive expectations of pleasure and sexual reward, but may also include the learning of negative expectations (Ågmo, 1999).

For most individuals, this sexual learning develops and evolves without problems. However, from the early days of sex research on it is recognized that the accidental pairing of an 'abnormal' stimulus with sexual arousal or orgasm can be 'at base' of the development of sexual deviations (Krafft-Ebing, 1892). Conversely, aversive sexual learning, as in absence of expected sexual reward (e.g. failure of orgasm) or in negative sexual experiences (e.g. painful sexual intercourse, sexual assault), may also contribute to the development of sexual dysfunction, since this may result in less attraction to incentives (Both et al., 2008a). As a consequence, the quantity and quality of incentives that can activate the sexual system depend on the individual's sexual history and genetic makeup (e.g. hormones and neurotransmitters), and differ from one individual to another. Although it is unclear at present if men and women differ with respect to basic sexual learning, it is speculated that women are more sensitive to variations in social and cultural factors (i.e., exhibit more 'erotic plasticity') compared to men (Baumeister, 2000; Toates, 2009, 2014). In women, a sexual stimulus tends to trigger a wider range of cognitions as compared to men (Laan & Janssen, 2007; Toates, 2014). Therefore it is suggested that women's sexual motivation and arousal might be more strongly controlled by cognitive factors,

whereas men's sexual motivation tends to be more strongly controlled by stimulus factors.

The individual variability in sexual responsiveness can be explained by the Dual Control Model based on the interaction between sexual excitatory and sexual inhibitory processes in the brain (Bancroft & Janssen, 2000). According to this model, the neurobiological inhibition of sexual response is of adaptive value, since it reduces the likelihood of sexual response and recognizes the distracting effects of sexual arousal occurring in situations when sexual activity would be disadvantageous or dangerous, or would distract the individual from dealing appropriately with other demands of the situation. The model can also explain why, even though the propensities of sexual inhibition and sexual excitation are adaptive and non-problematic for the majority, individuals with an unusually high propensity for excitation or a low propensity for inhibition are more likely to engage in high-risk or otherwise problematic sexual behaviours. Or likewise, the model can explain why individuals with a low propensity for sexual excitation or a high propensity for sexual inhibition are more likely to experience problems with impairment of sexual response. Moreover, according to this Dual Model, the (excitatory and inhibitory) effect of sexual stimuli are mediated by psychological and neurophysiological characteristics of the individuals involved, influenced by both genetic factors and learning history.

1.4. Dopamine

The mesolimbic DA system is crucially involved in aversive and appetitive motivational processes underlying learning and the execution of goal-directed behaviour (Robbins & Everitt, 1996; Robinson & Berridge, 2003). Research has demonstrated appetitive - aversive interactions in DA neurons in the brain reward system: when a neuron is excited by an aversive stimulus it is inhibited by an appetitive stimulus or vice versa (Bouton & Peck, 1992; Matsumoto &

Hikosaka, 2009; Nasser & McNally, 2012) and recruitment of the relevant motivational system -appetitive or aversive- is dependent on the rewarding or aversive stimulus. According to the incentive salience or 'wanting' hypothesis of DA, the mesolimbic dopamine system attributes incentive salience to representations of stimuli that were associated with appetitive reward (Berridge & Robinson, 1998). With other words, DA is hypothesized to be necessary for converting a neutral stimulus into an attractive 'wanted' stimulus that is capable of eliciting approach response (Berridge, 2007; Flagel et al., 2010). Rewards like food, drugs and sexual stimuli, have the ability to stimulate mesolimbic DA neurons in the human brain reward system, projecting from the VTA to the NAc, and increased extracellular concentrations of mesolimbic DA are implicated in responding for conditioned reinforcers (Berridge, 2007; Georgiadis & Kringelbach, 2012; Hamann et al., 2004; Oei et al., 2012; Pierce & Kumaresan, 2006; Richard et al., 2013; Rupp & Wallen, 2008). Hence, exposure to (conditioned) sexual cues activates reward regions, probably because of learned expectancies that the observed stimulus will provide genuine sexual reward (Heatherton & Wagner, 2011). The NAc activation is modulated by DA signalling (Richard et al., 2013), with higher activations in response to sexual reward cues when DA activity is increased, and lower activations when DA activity is decreased (Oei et al., 2012). Moreover, also relations between dopaminergic activity and the tendency to approach sexual stimuli have been demonstrated (Both et al., 2005). However, despite the substantial amount of research that has identified mesolimbic DA neurotransmission as essential in reward learning (Kringelbach & Berridge, 2009), to date no human research has been conducted on the role of DA in human sexual reward learning, while facilitation as well as impairment thereof is relevant in the context of treatment of sexual motivation disorders. Evidence for the possibility to block sexual reward learning by DA antagonists may open up new ways to treat compulsive sexual behaviour.

1.5. Extinction and Renewal of Conditioned Responding

As environments are continuously changing, the ability to flexibly readjust sexual learning such that it appropriately tracks the ongoing change in circumstances (e.g. a stimulus might cease to signal sexual reward while another becomes sexual rewarding) is of great importance. Generally, when the CS is repeatedly presented without the US, and the CS no longer predicts the aversive or appetitive outcome (Delemater, 2004), this will yield in a loss of conditioned responding. This process, known as *extinction*, has obviously clinical relevance, since it is thought to be the core mechanism of therapeutic interventions such as cue-exposure therapy (Hermans et al., 2006; Rescorla, 2001). In such therapeutic protocols, conditioned responses are lessened or inhibited by repeated or prolonged exposure to a cue (the CS) in absence of the event it used to predict (the US). This results in a decrease in the magnitude or frequency of the conditioned response (CR). As a result of classical conditioning, a CS can also acquire the hedonic valence of the US. This form of learning involves the transfer of affective value to an initially neutral stimulus as a result of its contingent presentation with (dis)liked stimuli, and is called evaluative conditioning (de Houwer, Thomas & Baeyens, 2001; Hermans et al., 2002). While in classical conditioning the CS elicits a US expectancy and CR (i.e. signal learning), in evaluative learning it is thought that the CS automatically evokes the representation of the US (Díaz, Ruiz & Baeyens, 2005). Research suggests that although extinction procedures do eliminate the expressions of US expectancy, extinction procedures do not change the expressed valence of a CS, and as a result, exposure treatment is often unsuccessful in reducing acquired subjective (dis-) likes (Baeyens, et al., 1992; De Houwer, Thomas & Baeyens, 2001).

It is speculated that humans are not only capable in coding events and/or stimuli that are related, but also in coding *how* these are related. According to De Houwer (2009), the persistence of evaluative learning effects

can be explained by the assumption that once a stimulus has been categorized as potential cause of an aversive or appetitive outcome, individuals fall back on their prior propositional knowledge about causal relations, including the general knowledge that causes tend to have additional effects. Former studies on conditioned sexual response have indicated that conditioned genital responses and subjective affect do not extinguish (Both et al., 2008b; 2011), suggesting resistance to extinction of appetitive and aversive conditioned sexual response. This is highly clinically relevant, because when conditioned valence and possibly genital arousal are relatively resistant to extinction procedures, then a combination of extinction with some other intervention (e.g. counter-conditioning) would presumably be more effective than extinction alone in the treatment of paraphilia, hypersexuality and related sexual disorders. Conversely, for the treatment of female sexual interest/arousal disorder, knowledge about how sexual responses can be effectively conditioned, or how (negative) conditioned subjective affect may decrease is highly relevant. Moreover, historically, theories of emotion have not given much consideration to sex. Despite the fact that it will likely yield important knowledge about mechanisms underlying sexual motivation and related sexual disorders, there is only limited empirical research on conditioning of sexual arousal, and research on sexual extinction learning in humans is even scarcer. Studies on sexual arousal responses involving women have been much scarcer than studies involving men for most of the history of sex research. Only a few studies have addressed sexual conditioning in both sexes (Hoffmann, Janssen & Turner, 2004; Klucken et al., 2009), and none of them have examined extinction of -aversively and appetitively- conditioned sexual responses systematically, using extensive extinction trials. As a result, it is not only unclear if sexually conditioned valence and possibly genital arousal are indeed relatively resistant to extinction procedures, but it is also unknown if there are gender differences in both acquisition and extinction of conditioned sexual arousal and response.

Additionally, it is known that many individuals relapse after 'successful' cue exposure treatments. Although CS-alone presentations may extinguish conditioned responses, the extinction procedure does not seem to erase the originally learned CS-US association. This original association is retained (Bouton, 2004). It is thought that contexts play an important role in regulating responses and in related relapse behaviour (Bouton, 2002, 2004). Renewal is the term used to describe recovery of extinguished behaviour as a result of context change (Bouton, 2004). Renewal is the restoration of conditioned responding in context A but not in context B when learning occurred in context A and extinction in context B. Despite its relevance for cue exposure based treatment strategies for learned maladaptive sexual responses, little attention has been given to this phenomenon of *renewal* of conditioned responding in appetitive conditioning, and research on renewal of conditioned sexual responses is even completely lacking in the literature.

Speculatively, based on studies on aversive and appetitive conditioning paradigms (Effting & Kindt, 2007; Vansteenwegen et al., 2005; Van Gucht et al., 2008) which have demonstrated renewal of conditioned responding as a result of context switch after extinction, it would be highly beneficial to generalize extinction procedures to other contexts and with multiple stimuli in order to reduce renewal of conditioned responding (i.e. relapse). However, since it is evidently impossible to cover all sorts of situations or stimuli in therapy sessions that patients might encounter in the future (Todd et al., 2014), any pharmacological agent that can render extinction context independent may provide an innovative method to reduce cue-induced relapse in the treatment of problematic reward-seeking behaviours. The glutamatergic N-methyl-D-aspartate (NMDA) receptor is essential in learning, memory (Reichelt & Lee, 2013). D-cycloserine (DCS) is a partial agonist at the NR1 NMDA receptor subunit and has been shown to enhance acquisition, consolidation, extinction and reconsolidation in several -especially aversive- associative learning

paradigms in rodents and humans (Kalisch et al., 2009; Myers & Carlezon, 2012; Torregrossa et al., 2013). There is some evidence in humans that DCS facilitates extinction of fear during cue– exposure therapy for a range of anxiety disorders (Fitzgerald et al., 2014), and limited studies have investigated DCS in the treatment of substance-dependent subjects, with mixed results (Myers & Carlezon, 2012; Reichelt & Lee, 2013). Nevertheless, evidence for clinical efficacy of DCS in exposure therapy for nicotine and cocaine addiction (Santa Ana et al., 2009; Price et al., 2013) provides a rationale for further investigation. Enhancing sexual extinction memory by means of a partial NMDA-receptor agonist, such as DCS, has not been studied, while is highly relevant in the context of treatment of for instance hypersexuality and related disorders.

1.6. Cognitive Emotion Regulation

Cognitive emotion regulation refers to the higher level processes by which individuals *intentionally* can regulate or modulate the intensity and direction of the physiological, behavioural, and experiential components of emotional responses via prefrontally mediated inhibition of subcortical response-related regions of the brain (Gross & Thompson, 2007; Frank et al., 2014). For instance, cognitive regulation can change or control emotion, as individuals may re-evaluate an emotion-evoking stimulus, or shift their focus of attention to diminish an undesired emotion (Gross, 1999). Making use of emotion regulation strategies, like generating, maintaining, decreasing (down-regulation) or increasing (up-regulation), humans are able to make changes in one or more of the various components (or response systems) of emotion (Aldao, 2013; Gross, 1999, 2002; Gross & Thompson, 2007). Emotion regulation strategies can influence emotions at the input phases (i.e. antecedent focused like cognitive reappraisal or attentional deployment) and at the output phase (i.e. response focused like suppression) (Gross, 1998; Webb, Miles & Sheeran, 2012). Gross and Thompson (2007) suggest that antecedent-focused strategies

(e.g. attentional deployment) are more effective than response-focused strategies. As relatively few studies on negative emotions and even less studies on positive emotions have investigated the effects of the promising active distraction strategies (where the emphasis is on participants to bring to mind something unrelated to the emotion or emotional stimulus to serve as a distraction), especially on behavioural and physiological measures of emotion, this is an important avenue for future research (Webb, Miles & Sheeran, 2012). Failures in the deployment of top-down cognitive control mechanisms or overactive bottom-up processes are thought to contribute to several forms of psychopathology (Heatherton & Wagner, 2011; Ray & Zald, 2012), including sexual disorders (Bancroft & Janssen, 2000; Both, Laan & Everaerd, 2011; van Lankveld, van den Hout & Schouten, 2004; Salemink, van Lankveld, 2006). Even though most research has been done into the urge to use drugs, fear and depression, the principles of cognitive emotion regulation would seem to be generally applicable to other domains (Beauregard, 2007; Heatherton and Wagner, 2011). Despite the hypothesized importance of understanding how to regulate or control the positive feelings associated with reward expectation, no research has been done on the influence of emotion regulation strategies on the expectation of sexual reward. Additionally, cognitive behavioural therapy (CBT) based on associative learning principles has emerged as the psychological treatment of choice for disorders in sexual interest and desire (Basson, 2005; Laan & Both, 2008; Both, Laan & Schultz, 2010; Trudel et al., 2001). CBT encompasses cognitive techniques such as cognitive restructuring of negative and/or sexually inhibiting thoughts, and behavioural techniques such as sex therapeutic exercises to (re)create different, more varied, or prolonged sexual stimulation to enhance sexually pleasurable experiences. It is thought that the interaction with pleasurable sexual stimuli and events desensitizes possible negative associations and facilitates sexual response acquisition and maintenance, and that memories of positive sexual experiences result in

expectations of sexual reward, which may subsequently enhance sexual interest and arousal (Basson, 2005; Laan & Both, 2008; Both, Laan & Schultz, 2010). It is likely that cognitive and behavioural processes interact during CBT. This makes clear that psychotherapy provides instructive examples of how cognitive, volitional intention 'regains' control over dysregulated emotions. However, research on the influence of emotion up-regulation on the expectation of sexual reward is lacking in the literature, despite the fact that insight in the mechanisms of these cognition-emotion interactions can help in the development of effective CBT interventions. Moreover, very little is known about possible gender differences in emotion regulation (Whittle et al., 2011), especially with respect to the regulation of positive emotions, including sexual arousal.

1.7. Stress and Sexual Emotion Regulation

Even though individuals can cognitively alter emotional responses to foster more adaptive responses and behaviour, people may fail to do so. Whether we are arguing intensely with a loved one, moving to a new city, or having problems at work, controlling emotions and related response when circumstances become stressful can be quite a challenge. Research has shown that participants who attempt to regulate their responses to reward cues (e.g. money, food or drugs), show increased activity in regions in the PFC associated with self-control and reduced cue-reactivity in region associated with reward processing (Heatherton & Wagner, 2011; Kober et al., 2010; Volkow et al., 2010). This indicates that regulation of appetitive responses requires top-down control of the brain reward system by higher-level control regions, such as the PFC. Self-regulation failure may occur when frontal executive functions are compromised, by for instance acute stress. One potential reason for this mental challenge posed by acute-stress might be that the presence of stress can decrease the weight of high-level top-down control (from the frontal brain

structures) and increase the weight that is exerted at a low level (i.e. subcortical regions involved in reward and emotion) (Heatherton & Wagner, 2011; Raio et al., 2013; Toates, 2014). Moreover, although the dopaminergic pathways are widely known for their involvement in the signalling of rewarding stimuli, as previously mentioned, also aversive events including acute stress, can activate the dopaminergic neurons in the brain reward system (Oei et al., 2014). The rapid cognitive effects of stress are thought to be mediated by neuroendocrine responses to acute stress exposure (i.e. increased cortisol levels) that impact not only upon subcortical reward structures (Oei et al., 2014) but also upon the functional integrity of PFC (Raio et al., 2013). Although negative affect has been long proposed to play an important role in the failure to exert self-regulatory control over thoughts and behaviour (Heatherton & Wagner, 2011), including risky sexual behaviour (Bousman et al., 2009), and the influence of stress on cognitive control of negative emotion as well as cocaine craving has been demonstrated (Raio et al., 2013; Sinha, et al., 2005), a direct relationship between the physiological stress response and the cognitive control of sexual arousal has not yet been examined. The findings from earlier studies on acute stress and emotion regulation suggest an important, yet unexplored, paradox: top-down regulation may be ineffective in mediating sexual responses precisely when such control is needed most. Moreover, it is important to keep in mind that, as mentioned before, the principles underlying cognitive regulation also form the basis of CBT. Therefore, the success of CBT relies on the availability of cognitive resources and intact executive function (Heatherton & Wagner, 2011; Hofmann, Schmeichel & Baddeley, 2012; Ochsner, Silvers & Buhle, 2012).

1.8. Outline of this Thesis

This thesis encompasses several studies on sexual reward learning and one functional magnetic resonance imaging (fMRI) study on the influence of acute-

stress on deliberate emotion regulation during the processing of sexual stimuli. To study behavioural and neurobiological influences on human sexual incentive learning, in all sexual conditioning studies described in this thesis (chapters three to nine) a differential conditioning paradigm was applied. Former studies on sexual conditioning using this paradigm demonstrated highly consistent findings on the impact of appetitive and aversive associative learning on sexual response (Both et al., 2008a,b; 2011). In the studies on appetitive sexual conditioning, neutral pictures served as CSs and genital vibrostimulation as US. In the aversive conditioning study, a painful electric stimulus at the wrist served as US, and two erotic pictures served as CS. At all times, only one CS (the CS+) was followed by the US during the acquisition phase. Physiological sexual responses (i.e. penile circumference in men and vaginal pulse amplitude in women) were assessed and subjective responses (i.e. ratings of subjective affect, subjective sexual arousal and US expectancy) were obtained. Except the chapters three and ten, in all studies a stimulus response compatibility task was included to assess automatic approach and avoidance tendencies towards the CSs. The first section of this thesis will focus on classical conditioning of sexual arousal response and related phenomena. With other words, this first section mainly focuses on low-level control processes involved in sexual reward learning. The second section of this thesis focuses on high-level control processes in sexual reward learning and sexual processing.

Section 1

In **chapter two**, a thorough review is given of animal and human studies that examined the role of classical conditioning, learning, and DA in sexual behaviour, which were published or in press before October 2013. This section will provide a background into understanding the role of associative learning in both normal and maladaptive sexual arousal disorders. Although the involvement of other neurotransmitter systems such as the serotonergic,

endorphin, and glutamate system in sexual behaviour has been reported, this chapter has a strong focus on the dopaminergic system.

Chapter three describes the experimental study of inhibiting dopaminergic tone in sexual reward learning. At present, research on the role of DA in human sexual reward learning is lacking, while facilitation as well as impairment of sexual reward learning is relevant in the context of treatment of disorders in sexual motivation. In this study, making use of a double-blind, parallel-conditions, placebo controlled design, it was investigated whether DA antagonism attenuates classical conditioning of sexual response in women.

In **chapter four**, an experimental study on extinction of appetitive conditioned sexual response is described. Earlier studies on conditioned sexual response demonstrated that women's conditioned genital responses and subjective affect did not extinguish during a brief extinction phase (Both et al., 2008b; Both et al., 2011). In this chapter a possible *resistance to extinction* of appetitively conditioned sexual response in both sexes is examined using extensive extinction trials. Likewise, **chapter five** describes a parallel study on extinction of aversively conditioned sexual responses in healthy men and women.

In **chapter six** a study on extinction and renewal of appetitively conditioned sexual responses in sexually functional men and women is described. Like mentioned before, despite its relevance for extinction-based treatments, studies on extinction and renewal in the human sexual domain are completely lacking.

In **chapter seven** a study on glutamatergic NMDA-receptor agonism in sexual reward learning is reported. Since conditioned sexual responding may be susceptible to renewal, a highly promising perspective is to investigate processes that modulate contextual processing during extinction procedures. Since the NMDA-receptor is considered essential for long-term potentiation, a process that underlies learning and extinction (Reichelt & Lee, 2013), an

investigation is reported on whether administration of DCS can enhance appetitive extinction memory and reduce the context specificity of extinction of sexual reward-associated cues in humans.

Section 2

Because research on the influence of emotion regulation on the expectation of sexual reward is lacking in the literature -in spite of the fact that insight in the mechanisms of these cognition-emotion interactions can help in the development of effective CBT interventions- in **chapter eight** a study on the influence of an emotion-down-regulation strategy on sexual conditioned responses and extinction thereof in healthy men and women was examined. Likewise, in **chapter nine** an investigation of the efficacy of an emotion up-regulatory strategy with expectations elicited by conditioned appetitive sexual stimuli. Moreover, in both chapters possible gender differences in the regulation of sexual reward are examined.

In **chapter ten** the influence of acute-stress on deliberate emotion regulation during the processing of sexual stimuli is investigated. Imaging research can help shed light on the way (acute) stress mediates the neurobiological reactivity to sexual stimuli, and whether this is dependent on stress-induced cortisol levels. Therefore, in this chapter the relationship between stress and sexual emotion regulation is reported, by examining the effect of acute-stress induced cortisol responses on within-subject functional activity in brain regions associated with sexual reward (e.g. the amygdala and NAc) and frontal regions during cognitive down-regulation of sexual arousal.

Finally, in the discussion we will aggregate and discuss our study findings and place them in an appropriate theoretical context. The discussion will be continued with addressing limitations of our work and discussing remaining challenges and further avenues for future research endeavours. Also possible clinical implications of our studies will be addressed.

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SECTION 1

Chapter 2

The Role of Conditioning, Learning and Dopamine in Sexual Behaviour:

A Narrative Review of Animal and Human Studies

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Abstract

Many theories of human sexual behaviour assume that sexual stimuli obtain arousing properties through associative learning processes. It is widely accepted that classical conditioning contributes to the aetiology of both normal and maladaptive human behaviours. Despite the hypothesized importance of basic learning processes in sexual behaviour, research on classical conditioning of the sexual response in humans is scarce. In the present paper, animal studies and studies in humans on the role of Pavlovian conditioning on sexual responses are reviewed. Animal research shows robust, direct effects of conditioning processes on partner- and place preference. On the contrast, the empirical research with humans in this area is limited and earlier studies within this field are plagued by methodological confounds. Although recent experimental demonstrations of human sexual conditioning are neither numerous nor robust, sexual arousal showed to be conditionable in both men and women. The present paper serves to highlight the major empirical findings and to renew the insight in how stimuli can acquire sexually arousing value. Hereby also related neurobiological processes in reward learning are discussed. Finally, the connections between animal and human research on the conditionability of sexual responses are discussed, and suggestions for future directions in human research are given.

2.1. Introduction

It is widely accepted that associative learning contributes to the aetiology of both normal and maladaptive human behaviours (Day & Carelli, 2007). Psychopathology and deviant sexual preferences and behaviours are also thought to develop through conditioning processes (Letourneau & O'Donohue, 1997; Ågmo et al., 2004; Martin-Soelch et al., 2007; Pfaus, 1999a,b; Singer & Toates, 1987; Toates, 2009). Knowledge about basic learning processes involved in sexual behaviours, can help foster potentially critical insights in the aetiology of extreme forms of sexual behaviour. This may help in the development of clinical treatments for those behaviours, including paraphilias, or deviant sexual preferences, that manifest perturbed motivation, but also for the more prevalent sexual desire and arousal disorders. Because much sexual behaviour is thought to be acquired through learning, and given the specific hypothesis that classical conditioning plays an etiological role in the development of sexual dysfunction, research in the area of classical conditioning of human sexual arousal is warranted. Despite the hypothesized importance of basic learning processes in sexual behaviour, research of classical conditioning of sexual response in humans is scarce (Akins, 2004; Pfaus et al., 2001). Sexual conditioning studies with women are even scarcer in the literature. In the present paper, we will discuss the studies that provide evidence for basic sexual learning and conditioning processes in sexual arousal, desire and behaviour, and focus especially on how stimuli can acquire sexually arousing properties.

According to incentive motivation models, sexual motivation is the result of the interplay of a sensitive internal sexual system with external motivational stimuli. External stimuli that can promote motivation are called incentive stimuli (Ågmo, 1999; Bindra, 1968, 1974; Both et al., 2007; Singer & Toates, 1987). Sexual incentives are generally positive, and likely to illicit approach behaviour. The motivational valence can be unconditioned (primary)

or conditioned (secondary) as a result of associative leaning (Di Chiara, 1995). Some stimuli, e.g. stroking or kissing may be innately sexually competent, but many sexual stimuli are not intrinsically sexually competent. The attractiveness of those stimuli depends on their history of positive or negative consequences and the resulting meaning that is stored in memory. Specific cues of sexually competent stimuli may gain learned incentive value through their association with the stimulus. While conditioned learning is not the only way in which humans acquire certain sexual behaviours, these processes represent an essential link between stimulus and response (Toates, 1998), and can be point of intervention in the treatment of disorders in sexual behaviour. Therefore, it seems highly valuable to gain insight in the processes through which stimuli may acquire sexual motivational value. Despite the overall consensus that learning plays an essential role in sexual development and the expression of sexual activity, sexual learning has only recently been tied into a more general framework of incentive motivation theory. Over the last decade, clinical and empirical support for the incentive-motivation model of desire has accumulated and the model is now incorporated into the current operational definition of sexual desire disorder in the DSM-5 (Brotto, 2010). This, combined with the fact that the model of incentive motivation was developed primarily with reference to research in nonhumans (Toates, 2009; Pfaus et al., 2001) makes clear that there is a need for an overview of the integration and extension of this theory with a focus on recent human sex research. Thereby, an attempt is made to form links between the processes described in the incentive motivation model and the proposed human brain regions involved.

First, we briefly discuss the different forms of conditioning and the associated neural systems. Further insights in associative learning by looking closely at the neural pathways involved in these processes have identified different brain regions and neurotransmitter systems involved in conditioned learning. Especially, dopaminergic terminations in the basal forebrain foster

sexual arousal and sexual motivation and seem to play a major role in reward learning (Di Chiara, 1999; Dominguez & Hull, 2005; Hull et al., 2004). Gaining knowledge about the functional neurobiology underlying human sexual behaviour is important, as this may lead to understanding of functions that apply to the most primitive aspects of human mental functioning. Thereby, it may also offer an opportunity for intervention. Although the involvement of other neurotransmitter systems such as the serotonergic, endorphin, and glutamate system in sexual behaviour have been reported, the current article primarily focuses on the dopaminergic system. Further on, we briefly review the literature on appetitive and aversive conditioning of the sexual response in animal studies. Mainly results from rat studies will be discussed, but when relevant, studies with other rodents, avian species, nonhuman primates and even fish will be discussed. Subsequently, the literature of sexual conditioning in humans is reviewed. In that section we will first discuss literature on the conditioning of the male sexual response, after which we will discuss the research conducted so far on the conditioning of the female sexual response. We will conclude with a discussion and suggestions for future directions. But first, for a thorough understanding, we will outline the different forms of conditioning and the related basic learning phenomena in the following section.

2.2. Basic learning processes

Why include basic learning mechanisms in an understanding of the neurobiology of sexual behaviour? From an evolutionary perspective, the development of sexual learning mechanisms is thought to offer some reproductive advantage and modulate reproductive fitness. This has indeed been shown by several animal studies. For instance, classical-conditioned male quail subjects released greater volume of semen and greater numbers of spermatozoa than control subjects (Domjan et al., 1998). Second, in blue gourami fish, classical conditioning provides the means to enhance territorial

defence and yields large reproductive benefits (Hollis et al., 1997). Classical-conditioned males are able to spawn with females sooner and produce more young than males that do not have the benefit of a signal. These examples demonstrated that animals can learn to associate environmental cues with the appearance of biologically important events. More important, the ability to anticipate the appearance of such different biologically important events suggests that learning principles may play a significant role in the behavioural ecology of all kinds of animals.

2.2.1. Habituation, sensitization, generalization and discrimination

Habituation is a systematic decrease in the magnitude of a response upon repeated presentations of an eliciting stimulus. This decrement is not due to fatigue or receptor adaption (Thompson & Spencer, 1966). Sensitization, in contrast, is the progressive amplification of a response after repeated administrations of a stimulus. An important characteristic of habituation is that it is stimulus-specific. For example, a habituated response can occur for the sound of a barking dog, without affecting the magnitude of the response to the sound of a slamming door. Yet, a transfer of habituation from one stimulus to a new, but equivalent stimulus is possible. This transfer and other transfers of the effects of conditioning to similar stimuli in general are covered by the term generalization. The amount of generalization depends on the degree of similarity between the stimuli (Mazur, 2002). The opposite of generalization is discrimination, in which it is learned only to respond to one stimulus but not to a similar stimulus. The degree of habituation depends on multiple factors such as frequency of stimulation, stimulus duration, stimulus intensity and the number of previous habituation trials (O'Donohue & Geer, 1985). In male animal subjects, a habituation-like phenomenon is detected when repeated copulations reduce sexual interest in a familiar female partner, but introduction of a novel receptive female sometimes results in recovery of the sexual

behaviour (Domjan & Hollis, 1988). Second, several researchers have reported findings that support short-term habituation of sexual arousal in humans (Koukounas & Over, 1993; Meuwissen & Over, 1990). Habituation effects can both be temporary as they can be retained for very short periods of time and be of a long term character. Recovery of the initial response can be observed after a longer time course. We will discuss this phenomenon of recovery in more detail later.

2.2.2. Classical conditioning

Classical conditioning or also called Pavlovian conditioning is one of the most studied paradigms in behavioural psychology and has a long and distinguished history dating back to pioneers in this field like Pavlov, Thorndike and Skinner. This form of associative learning has been demonstrated in a wide range of species and response systems (Hollis, 1997; Turkkan, 1989). Pavlovian responses, mainly involving approach and withdrawal, are elicited by the appetitive or aversive valence associated with predictive stimuli (Bouton, 2006). Biological relevant outcomes such as food can be labelled as unconditioned stimuli (US), because they are able to evoke innate or unconditioned responses (UR), such as salvation, approach and consumption (Pavlov, 1927). Through the repeated associating of a neutral stimulus (NS) with an US, the NS will eventually trigger the same reaction as the US (Pavlov, 1927; Bindra, 1974; Mazur, 2002; Pfaus et al., 2001). For instance, research has shown that in a 'triad mating paradigm' with two sexually receptive females, male rats trained previously to associate a neutral odour (almond or lemon) with sexual activity ejaculated preferentially with females bearing that odour (Pfaus, 1999a,b). The NS is now called the conditioned stimulus (CS) and the reaction to the CS is called the conditioned response (CR). According to classical models of learning, CS-US pairing causes the CS to become an 'emotional substitute' for the US.

Subsequently, the CS ultimately elicits similar emotional responses as the US does (Blair et al., 2005).

2.2.3. Basic principles of classical conditioning

Contiguity, contingency and prediction error are three factors that govern classical conditioning (Mazur, 2002; for a review see Schultz, 2006). Contiguity refers to the requirement of near simultaneity, which means that a US needs to follow a CS or response by an optimal interval of time. Contingency refers to the requirement that a US needs to occur more frequently in the presence of a stimulus as compared with its absence. Furthermore, conditioning will only occur when the CS is predictive of something important, such as an upcoming reward or shock. Prediction error postulates that even when it occurs in a contiguous and contingent manner, a reward that is fully predicted does not contribute to the process of learning. This means that learning advances only to the extent to which a reinforcer is unpredicted and the process of learning slows as the reinforcer becomes more predicted. Furthermore in extinction learning, the CS is no longer paired with the US, so conditioned behaviour diminishes (Delamater, 2004; Mazur, 2002; Schultz, 2006). As a result, the ability of the CS to evoke the CR is decreased. However, extinction does not result in complete unlearning of the CS-US association (Bouton, 2004; Delamater, 2004; Mazur, 2002; Rescorla, 2001). Phenomena like reinstatement, spontaneous recovery and renewal of conditioned responding can occur following a context shift out of the extinction context. Reinstatement is the restoration of CR in the context where extinction training occurred but not in a different context after an US is presented again. Renewal is the restoration of the CR in context A but not in context B when learning occurred in context A and extinction in context B, and spontaneous recovery can be described as the restoration of the CR when the retention test occurs after a long but not a short delay after extinction training. Extrapolating to clinical practice, someone who

acquired a craving for internet-sex at home (context A) is successfully extinguished by cue exposure therapy in a therapeutic setting (Context B), may experience strong craving upon changing context such as sitting behind the computer at home (Context A) or a different computer in a new situation (Context C). So it appears that specific CS-US associations are preserved after an extinction treatment. Moreover, extinction procedures seem to result in new learning that is especially context-dependent (Bouton, 2004) and this can be formulated in terms of the formation of an inhibitory association between the CS and the former CR (Delamater, 2004).

Furthermore, not all stimuli will result in equally rapid learning. This brings us to the phenomenon of preparedness. The fundament for the assumption of preparedness comes from Seligman (1970), who in the context of fear learning suggested that primates are predisposed to condition fear more readily to stimuli related to recurrent survival threats than to stimuli that never have threatened survival or to fear-relevant stimuli that emerged only recently in our evolutionary history. As with prepared associations for the module of fear, one can postulate that this principle also accounts for appetitive modules, like that of sexual arousal. It has been hypothesized that humans indeed are born with sensitivity to what we call sexual stimuli (Everaerd et al., 2000; Janssen et al., 2000) and may be prepared to form particular associations between stimuli and sexual arousal (Laws & Marshall, 1990). For example, an abdomen of the opposite sex has more sexual relevance than a penny jar for instance, and should therefore become more easily associated with sexual arousal. The theory further proposes that prepared associations should be easy to acquire and also obey different laws of learning than do nonprepared associations. Prepared associations will be more resistant to extinction relative to nonprepared associations and are less influenced by rational or cognitive input (Seligman & Hager, 1972; Öhman & Mineka, 2001). Although an extensive and critical discussion of the literature on preparedness is beyond the

scope of this article, it is worth mentioning that alternative theories to explain resistance to extinction have arisen. Lovibond et al. (1993) proposed an alternative theory of selective sensitization, where a pre-existing response tendency is activated by a perceived threat. The interpretation of the studies by Mineka and colleagues has also been questioned by Davey (1995), who suggests that the readiness with which some stimuli become associated with aversive outcomes arises from biases in the processing of information about threatening stimuli rather than from phylogenetically based associative predispositions. Nevertheless, despite these critiques, the preparedness theory is still widely accepted as a valid account of the aetiology of fears and phobias. Concerning sexual learning, the theory would imply sexual relevant cues to become more easily associated with sexual arousal, and thereby prepared sexual associations will be more resistant to extinction.

2.2.4. Operant conditioning

The learning processes in classical conditioning are centred on the contingency between two stimuli, whereas those involved in operant conditioning are centred on the contingency between a stimulus and a response. However, despite these differences, it is not always easy to distinguish whether classical or operant responses are being observed in an experimental situation. The central mechanism underlying the mechanism of operant conditioning, also referred to as instrumental conditioning, is reinforcement, and was introduced by Skinner (1937). In operant conditioning an organism learns a new motor response in order to obtain a positive outcome (e.g. food, mating opportunities or the avoidance of pain). For instance, male rats can be taught to run from level to level in bilevel chambers in anticipation of receiving access to a receptive female (Pfaus et al., 1990). Operant conditioning focuses on changes in the frequency of (goal-directed) behaviour, resulting from its association with reinforcing or punishing consequences. Reinforcing stimuli, such as rewards,

have the power to enhance the probability of occurrence of a response. This means that rewarding stimuli or events can reinforce behaviours by strengthening associations between stimuli and behavioural responses and Thorndike (1911) described this as 'Law of Effect': the greater the satisfaction or discomfort, the greater the strengthening or weakening of the association.

2.2.5. Rewards and reinforcers

Although the terms reward and reinforcement are frequently used interchangeably, it should be mentioned that both are clearly different. Something that is reinforcing is not necessarily rewarding (Di Chiara, 1995, 1999). From a purely behaviourist point of view, and as described before, reinforcement refers to an increase in the probability of a response (Paredes, 2009). This increase can be associated with the introduction of an appetitive stimulus or with removal of an aversive one. Secondly, it can be measured as the strength of a behavioural response (Berridge & Robinson, 1998). Punishers act as reinforcers by increasing behaviour that result in decreasing the aversive outcome. In general, reinforcers are stimuli that strengthen the association of events to which they are contingent. In contrast, reward refers to the ability to elicit an approach behaviour, similar to incentive motivation. And to be complete, an incentive can be defined as any stimulus that activates approach behaviour (Schultz et al., 1997). Aversive environmental stimuli, such as loud noises or painful shocks, have opposite valence to reward and induce withdrawal behaviour (Schultz, 2006). They act as reinforcers by increasing and maintaining avoidance behaviour on repeated presentation, thereby reducing the level of danger or impact of damaging events. Furthermore, aversive stimuli or events induce emotional states of anger, fear and panic. Food, water and certain kinds of sexual stimulation are called primary rewards, because they do not require associative learning processes as they can reinforce behaviour (Di

Chiara, 1999; Schultz, 2006; Wise, 2002). On the contrary stimuli like money gain their reward value by learned associations with primary rewards.

2.2.6. Incentive motivation and associative learning in sexual behaviour

Within the incentive motivational framework (Bindra, 1974; Robinson & Berridge, 2003; Toates, 2009), incentives that predict sexual reward can elicit a conditioned appetitive motivational state, reflected in sexual arousal and sexual desire. Common to most theories on sexual functioning is the assumption that sexual behaviour is intrinsically rewarding or reinforcing (Ågmo, 1999; O'Donohue & Plaud, 1994; Woodson, 2002). Especially ejaculation or orgasm, can be seen as primary rewards, and therefore can reinforce learning processes (Ågmo et al., 2004; Tenk et al., 2009). The positive affect produced by sexual encounters can become associated to environmental stimuli, and consequently, these stimuli can become conditioned sexual incentives and thereby come to elicit sexually relevant responses. The strength of the conditioning will be enhanced by repeated exposure to the specific stimuli while experiencing sexual reward. However, when expected rewards are absent this may have aversive consequences, which may become associated to environmental stimuli resulting in a decrease in responding or approach behaviour (Ågmo et al., 2004). Consequently, the quantity and quality of incentives that are able to activate the sexual system depend on the individual's sexual learning history and varies between individuals. For instance, some individuals may never form associations between sexually rewarding experiences and stimuli or only to a limited amount, in which case there will be a limited number of incentives that can activate sexual arousal and desire. Under other conditions, there may be a history of strong and frequent associations, resulting in a large number of stimuli that can elicit sexual arousal and desire. Also, associations may occur in conjunction with deviant stimuli and lead to a paraphilia (Geer et al., 1993; Toates, 2009).

2.3. Neurobiological processes in reward

2.3.1. The reward system

The first authors to describe brain reward pathways were Olds and Milner (1954). They observed that when they placed electrodes in certain brain areas of rats, these rats proceeded to active self-stimulation of these brain areas. Since then, a substantial amount of research has found a common pattern of activation in brain areas that respond to diverse rewarding stimuli. Both animal studies and human studies have shown that rewards like food, sex and drugs, have the ability to stimulate the mesoaccumbens dopamine projection within different brain areas and increase extracellular concentrations of mesolimbic dopamine (DA) (Arana et al., 2003; Di Chiara, 1998; Di Chiara, 1999; Giuliano & Allard, 2001; Hyman et al., 2006; Kalivas & Nakamura, 1999; Pierce & Kumaresan, 2006; Schultz et al., 1997, 2000; Schultz, 2006; Volkow et al., 2002; Damsma et al., 1992; Richard et al., 2012). Whereas DA responses distinguish rewards from non-rewards, DA neurons apparently do not discriminate between different sorts of reward like objects, food or liquid reward (Schultz, 2002). Although it is interesting to note that they do distinguish between a less-than-expected punishment and a greater-than-expected reward (Fiorillo, 2013). The observation that DA neurons are activated by all sorts of rewards has led to the hypothesis that the brain may process rewards along a single final common pathway in the form of a kind of common neural currency (Fields et al., 2007; McClure et al., 2004). The circuits involved in this process have been called the brain reward system. It is suggested that the reward pathway can be divided into two parts, i.e. the opioid ('liking') system and the dopaminergic ('wanting') system (Berridge, 1996; Di Chiara, 1995, 1998; Zhang & Kelley, 1997, 2000). Whereas the opioid system is associated with satisfying consummatory aspects of reward (e.g. blissfulness, sedation), the dopaminergic system is associated with the incentive and acquisition aspect of reward

(Berridge, 2007a,b; Di Chiara & North, 1992; Wightman & Robinson, 2002; Wise, 2002). The human brain has an exquisite sensitivity to signals for reward, by activation of this mesolimbic and mesocortical reward circuitry (Adinoff, 2004; Di Chiara, 1995, 1999; Koob, 2000; Schultz, 2000). The dopaminergic mesolimbic and mesocortical pathways originate with dopaminergic cell bodies in the ventral tegmental area (VTA) (Fields et al., 2007; Koob, 2000; Tanaka et al., 2004; Robbins & Everitt, 1996). This dopamine-rich nucleus is located in the midbrain, medial to the dopamine-rich substantia nigra and ventral to the red nucleus. The dopaminergic axons most extensively project to the nucleus accumbens (NAc), but also extend to other brain structures, like the septum, amygdale, prefrontal cortex (PFC), hippocampus and certain parts of the thalamus (Adinoff, 2004; Berke & Hymann, 2000; Di Chiara, 1995; Fields et al., 2007; Koob, 2000; McClure et al., 2004). The projection from the VTA to the NAc is the richest in DA neurons and is 65–85% dopaminergic (Fields et al., 2007). The DA system assigns incentive salience to percepts and representations (Berridge, 1996). In this way DA causes an event or environmental stimulus to become attractive and 'wanted', which can subsequently mediate approach behaviour.

Whereas DA in the NAc enhances motivation, DA in the lateral hypothalamus (LH) inhibits motivated behaviours (Parada et al., 1995; Hull, 2011). Research with rats with excitotoxic lesions of the lateral hypothalamic area and NAc revealed that the LH plays an inhibitory role in the regulation of sexual arousal and an excitatory role in the regulation of ejaculation. The NAc was found to play an excitatory role in the regulation of sexual arousal (Kippin et al., 2004). Research with female rats has shown that mesolimbic DA neurons that terminate in the NAc can be modulated in vivo by estrogen and that this modulation may be mediated by both genomic (long term) and nongenomic (short term) mechanisms (Thompson & Moss, 1994). Moreover, testosterone facilitates copulation in male rats by increasing neuronal nitric oxide synthase

immunoreactivity in the medial preoptic area (mPOA) of the hypothalamus, which in turn increases both basal and female stimulated DA release (Hull et al., 1995; Hull et al., 1997; Putnam et al., 2003). Glutamate is also released in the mPOA during copulation, and glutamate, acting via NMDA receptors and calcium inflow, may increase nitric oxide, and thereby DA release (Hull, 2011). In addition, research has demonstrated interactions between glutamate and gonadal steroids in the regulation of limbic and hypothalamic functions (Diano et al., 1997). This mechanism seems to be gender and site specific, suggesting that excitatory neurotransmission and related physiological mechanisms also may be distinctly different in males and females (Diano et al., 1997; Orsini, 1985).

Despite little is known about hormonal influence on the DA reward system in humans, research has demonstrated sex differences in striatal DA release in healthy men and women (Munro et al., 2006; Riccardi et al., 2006, 2011). For instance, Munro et al. (2006) demonstrated greater DA release in men compared to women after amphetamine administration in the ventral striatum, anterior putamen, and anterior and posterior caudate nuclei, whereas other researchers found greater DA release in extrastriatal areas in a similar study (Riccardi et al., 2006). Variations in dopamine-related genes and in hormone levels affect the physiological properties of the DA system in nonhuman primates and modulate the processing of reward and social information in humans (Caldú & Dreher, 2007). For instance, it is suggested that testosterone regulates incentive sensitivity through interactions with mesolimbic DA pathways (Wood, 2008; Hermans et al., 2010). And second, some observed sex differences in response to stimulants are in large part due to the fluctuations in estrogen and progesterone that occur over the female reproductive cycle. For example, several of the positive subjective effects of amphetamine (e.g. euphoria and increased energy) are potentiated during the follicular phase relative to the luteal phase (Justice & de Wit, 1999). These

findings help in the understanding of the biological mechanisms underpinning addictive behaviours and how these differentially affect vulnerability to drug abuse or the development of sexual dysfunctions in men and women.

2.3.2. The reward system in sexual behaviour

A number of investigations in humans using fMRI technique have consistently shown that sexual stimuli evoke neuronal activity in the reward system (Garavan et al., 2000; Park et al., 2001; Karama et al., 2002; Hamann et al., 2004). Using different techniques, studies have detected overlapping activation patterns across multiple brain regions (Fonteille & Stoleru, 2010), including ventral striatal regions involved in reward in both men and women in reaction to visual sexual stimuli (Hamann et al., 2004; Park et al., 2001; Ponseti et al., 2006; Stóleru et al., 1999; for a review see Rupp & Wallen, 2008) or during ejaculation or feelings of orgasm (Georgiadis et al., 2009; Holstege et al., 2003; Komisaruk et al., 2004; Komisaruk & Whipple, 2005; McClure et al., 2004). In the imaging study by Walter and colleagues (Walter et al., 2008) patterns of differential activation between several regions related to a brain network of sexual arousal were compared. They demonstrated that activations in the ventral striatum and hypothalamus were related to stimulus specific sexual intensity, and independent of induced general emotional arousal or valence. Activations in the anterior cingulate cortex were associated with an interaction between sexual intensity and emotional valence. Recent studies using high resolution fMRI indicated extension of this network to thalamic nuclei (Metzger et al., 2013). Studies on brain activation during orgasm (Georgiadis et al., 2009; Hamann et al., 2004; Karama et al., 2002; Holstege et al., 2003) show activation in the region of the VTA and NAc, suggesting they have a role in mediating orgasmic pleasure in humans. These findings presumably represent an anatomical substrate for the strongly reinforcing nature of sexual activity in humans. The ability of sexual behaviour, especially orgasm and ejaculation, to

increase the concentration of DA in the NAc is considered to be crucial to their reinforcing effects (for reviews see Berke & Hymann, 2000; Di Chiara, 1999; Volkow et al., 2002).

Furthermore, results from a study in our laboratory support the view that DA is involved in the energetic aspects of appetitive sexual behaviour, at least in men (Both et al., 2005). It was found that levodopa facilitates early motor preparation – as measured with reflex modulation – in response to sexual stimuli. And a more recent fMRI-study from our lab (Oei et al., 2012) provides compelling evidence for a mediating role of DA in processing of subconscious perceived sexual stimuli. It was found in healthy young men that levodopa significantly enhanced the activation in the NAc and dorsal anterior cingulate cortex in response to subliminal sexual stimuli, whereas haloperidol (a DA antagonist) decreased activations in those areas. This first evidence for pharmacological modulation of implicit sexual reward processing, points at the possibility for DA to affect sexual motivation at its earliest onset, that is, outside awareness. But as these results only apply for men, further studies are warranted to investigate the role of DA in female sexual behaviour.

There is agreement in the literature that androgens play a conditional role in sexual responsiveness (Bancroft, 2009), and from studies in rats there is evidence for an interaction between sex steroids and DA in the control of sexual behaviour (Hull et al., 1999). Gonadal steroids regulate dopaminergic innervation in both hypothalamic and extra-hypothalamic structures at various developmental stages in male rats (Hull et al., 2004). Moreover, there is a remarkable consistency across species in the role that the mPOA plays in the orchestration of consummatory sexual responses (Bancroft, 1999). In female rats, estradiol increases oxytocin levels and release in the mPOA stimulating the lordosis reflex (Caldwell and Moe, 1999). Moreover, research supports the hypothesis that a rise in DA in the mPOA is specifically related to sexual motivation in males as compared to copulatory behaviour per se (Hull et al.,

1995; Kleitz-Nelson et al., 2010a,b). Although, it appears that testosterone is necessary for mPOA DA release during male copulatory behaviour and for mating itself, testosterone alone does not elicit DA in mPOA (Wood & Swann, 1999). Research suggests that testosterone creates a permissive environment that allows external sensory stimuli to induce mPOA DA release during copulation (Dominguez et al., 2001; Dominguez & Hull, 2001). In line with this, studies in humans have shown that high levels of testosterone are associated with reward sensitivity, and it is suggested that testosterone regulates incentive sensitivity through interactions with mesolimbic DA pathways (Wood, 2008; Hermans et al., 2010).

2.4. Associative learning and DA

Considerable evidence exists regarding the role of DA in memory and learning (Berke & Hymann, 2000; Di Chiara, 1995, 1999). Research has shown that DA activity is associated with responses to novel stimuli; encoding reward function; error detection signalling during the acquisition of new learning; and approach behaviour and incentive motivation (Schultz, 1998; Schultz et al., 1997; Montague et al., 2004; Schultz, 2002). There is substantial evidence suggesting that mesolimbic DA plays a critical role in the interpretation of stimuli and the acquisition of behaviours reinforced by rewarding stimuli (Adinoff, 2004; Kirsch et al., 2003; Di Chiara, 1995, 1999; Koob & Bloom, 1988; Schultz, 1998). For instance, neurons in the VTA contribute to both positive reinforcement and to the acquisition and expression of learned appetitive behaviours (Everitt & Robbins, 2005; Fields et al., 2007; Kalivas & Volkow, 2005; Schultz, 2002; Wise, 2002). Also, conditioning of an otherwise neutral stimulus by repeated association with a certain stimulus can be reinforced by stimulation of DA transmission (Di Chiara, 1995; Schultz, 2002).

As mentioned before, incentive salience transforms the neural representations of conditioned stimuli, converting an event or stimulus from a

neutral representation into an attractive and 'wanted' incentive (Berridge & Robinson, 1998; Berridge, 2007a,b). Subsequently, learning hypotheses posit that DA neurons mediate associative learning and expectations based on previous experience with a stimulus. In order to connect the learned predictive significance of a cue with appropriate responses, storage of specific patterns of information within the brain is required. As a result, internal representations of the reward-related stimulus and series of cue-related action sequences are stored in memory. Furthermore, behavioural responses can increase with repeated exposure to a rewarding stimulus (Kalivas & Stewart, 1991), because strengthening of stimulus-response and stimulus-reward associations sensitize the mesolimbic pathways (Di Chiara, 1999). This sensitization has been proposed as a central neural mechanism underlying addiction disorders (Robinson & Berridge, 2003; Hyman et al., 2006). When drugs or natural rewards evoke an increased DA release from the VTA into the NAc, this further alters the responsiveness to glutamate. The VTA and NAc receive extensive glutamatergic inputs from the prefrontal cortex and other brain areas. These excitatory inputs are considered crucial for establishing addictive and other motivated behaviours (Chen et al., 2010). CREB (cAMP response element-binding protein) is a nuclear transcription factor, involved in the development of addictive behaviours (Wise & Morales, 2010; Nestler, 2001). In response to ingestion of drugs or in response to natural rewards, the DA levels especially in the NAc rise. This stimulates DA-responsive cells to enhance cyclic AMP (cAMP) concentrations, thereby activating CREB. CREB generates a specific gene expression that codes proteins. One of these CREB-dependent proteins is dynorphin. Dynorphin is synthesized in the NAc and is a natural molecule with opium-like effects. It triggers a negative feedback loop, exerting inhibitory effects on VTA neurons. But since CREB is switched off only shortly after drug consumption has ended, this transcription factor may not be responsible for relapse in chronic substance abuse or other addictive behaviour

(Esch & Stefano, 2004). Delta FosB is another transcription factor that exerts its functions in response to chronic drug abuse and is also released in NAc. Interestingly, delta FosB is also induced in response to repetitious non-drug rewards. For this reason, it is suggested that delta FosB represent a more general mechanism participating in reward-associated behaviour change. Delta FosB remains active for a very long period following drug ingestion or following natural rewards and therefore delta FosB may cause sensitization to drugs or natural rewards (Kelz et al., 1999; Nestler, 2001). Delta FosB exerts its effects on behaviour through the AMPA (-amino-3-hydroxy-5-methylisoxazole-4-propionic acid) glutamate receptor subunit GluR2 in the NAc. In addition, research has demonstrated that the LH facilitates glutamate-mediated responses, and regulates the glutamate-dependent long-term potentiation in VTA DA neurons. Orexins (or hypocretins) are neuropeptides made exclusively in the hypothalamus. Research has shown that a subset of these cells in the LH is involved in reward processing and addictive behaviours. Orexin-containing neurons from the LH project densely to the VTA (Borgland et al., 2008). Orexin facilitates activation of VTA DA neurons by stimulus-reward associations. This LH-VTA orexin pathway was found to be necessary for learning a morphine place preference. These findings are consistent with results showing that orexin facilitates glutamate-mediated responses, and is necessary for glutamate-dependent long-term potentiation in VTA DA neurons. Since LH orexin cells are an important input to VTA for behavioural effects associated with reward-paired stimuli, LH orexin neurons are thought to play an important role in reward processing and the development of addictive behaviours (Aston-Jones et al., 2010; Harris & Aston-Jones, 2006; Harris et al., 2005). In addition, gonadal steroids possibly differentially regulate pituitary orexin receptors and adrenal orexin receptors in male and female rats and may therefore contribute to specific sex-dependent neuroendocrine and endocrine actions of orexins (Jöhren et al., 2003). Hull and co-workers (Muschkamp et al.,

2007) suggest a model for the regulation of the orexin system by gonadal steroids, and VTA DA by the orexin system. It is suggested that estradiol, synthesized from gonadal testosterone by aromatase, acts on estrogen receptors containing neurons in the bed nucleus of the stria terminalis, mPOA, and LH. These structures project to hypothalamic orexin neurons. Orexin projections to VTA enhance midbrain DA neuronal activity during male sexual behaviour. This effect may be blocked by intra-LH infusions of serotonin that inhibit orexin activity, impairing sexual behaviour and NAc DA release.

2.5. The role of learning in the sexual behaviour of animals

2.5.1. Male studies

CS-characteristics: Early studies on sexual learning employed arbitrary auditory and visual stimuli as CSs. When these cues are paired with the presentation of a member of the opposite sex or with the opportunity to copulate, a rapid acquisition of conditioned approach to the stimuli is found in male animal subjects (Farris, 1967; Hollis et al., 1989; Ågmo, 1999; Akins et al., 1994; Burns & Domjan, 1996; Domjan et al., 1986). For instance, Farris (1967) used a buzzer as CS and access to a live female quail as US to classically condition strutting (i.e. courting behaviour) in male quail. And in a study by Domjan et al. (1986) conditioned approach behaviour was observed using a red light bulb as CS prior to access to a female quail. Conditioned males approached and remained near the CS and had shorter copulatory latencies than control subjects.

Next to auditory or visual stimuli, odours can also serve as CS. A substantial amount of research has demonstrated that when neutral odours like almond or lemon, are paired with copulation, male rats develop a conditioned ejaculatory preference (CEP) for females bearing the olfactory cue associated with copulatory training (Ismail et al., 2009a,b; Kippin & Pfaus, 2001a,b;

Kippin et al., 1998, 2001). Also in nonhuman primates, conditioning of sexual arousal has been established making use of olfactory cues. Snowdon and colleagues (2011) successfully conditioned male marmosets to copulation with a sexually receptive female using an arbitrary olfactory cue (lemon odour) as CS. Post-conditioning, males showed sexual responses (erections, and increased exploration of the location where they previously experienced a receptive female, increased scratching) to the olfactory cue in absence of any cues from a female. The CRs were demonstrated even up to a week after the end of the conditioning trials.

Furthermore, research has demonstrated that places can also become associated with sexual encounters and may influence CRs in animals. Both male rats and quails develop a conditioned place preference (CPP) for an environment associated with copulation with a receptive female (Ågmo & Berenfeld, 1990; Paredes & Alonso, 1997; Akins, 1998; Everitt, 1990; Pfaus et al., 2001; Tenk et al., 2009). Furthermore, Sachs & Garinello (1978) demonstrated that when male rats were placed into the same chamber in which copulation had previously occurred, the latency to display penile erections was reduced dramatically. Another remarkable study was conducted by Pfaus et al. (2013), in which it was found that somatosensory stimuli can also be used to condition sexual arousal in male rats. Males displayed faster intromission and ejaculation latencies when they were tested with a harness jacket on, which they wore during prior sexual experience.

Pfaus and colleagues have demonstrated that the association with reward can also reduce the impact of aversive stimuli (Pfaus et al., 2001). They gave rats their first nine sexual experiences with either cadaverine-scented females, or unscented control females. Cadaverine is an aversive odour, which is produced in decaying flesh. Therefore, cadaverine is considered unconditionally aversive. A third group of males were habituated to the cadaverine odour in their home cages and copulation trials with unscented

females. During the 10th trial all males from the three groups were allowed to copulate freely with two receptive females: one scented with cadaverine and one unscented. Both males in the control group and from the habituation-group pursued the unscented females selectively and ejaculated exclusively with them. In contrast, males that had copulated previously with cadaverine-scented females pursued, copulated, and ejaculated with both females. This indicates that the aversive properties of cadaverine had been diminished after pairing with sexual reward. This study demonstrated that an unconditionally aversive odour can be made less aversive and even conditionally appetitive by pairing with sexual reward.

CS-US similarity: Research has suggested that although arbitrary CSs such as auditory and visual stimuli appear to be effective CSs, more female-like CSs appear to elicit different CRs in male animals. In a study by Domjan et al. (1988) male quail acquired conditioned approach behaviour to both a yellow stuffed toy, and a female quail whose appearance had been altered by attaching bright feathers to her shoulders. However, only the female elicited conditioned copulatory responses. Furthermore, a CS that included species-typical cues affected the acquisition and extinction of conditioned sexual responses in male quail. Results suggest that conditioned responses toward sexually relevant CSs, in contrast to results obtained with arbitrary CSs, might be highly resistant to extinction in male quail. In line with former theories (Rescorla & Furrow, 1977), Krause et al. (2003) suggested that CS-US similarity is an important factor in conditioning.

CS-US intervals: Zamble et al. (1986) demonstrated delay learning in a sexual conditioning paradigm. Rats were placed for different time spans in a plastic tub that served as CS. A female rat served as US and was separated from the male rat by a divider. The duration of CS-US intervals was manipulated. After conditioning, male rats demonstrated a longer ejaculatory latency irrespective of the duration of the CS-US interval, in comparison with

ejaculation latency prior to conditioning. Thus, sexual learning occurred at varying and relatively long CS-US intervals. In an attempt to replicate the study of Zamble and colleagues in an avian species, Akins et al. (1994) separated male quail subjects from the female's smaller cage and used a grey foam block with bilaterally-attached orange feathers as CS. Quails receiving 0.5, 5 and 10 min CS-US intervals showed an increase in the percent time they spent near the CS. The results from this study were interpreted as evidence that also in avian species, sexually conditioned approach behaviour can occur with long CS-US intervals. Another noteworthy study by Villarreal and Domjan (1998) showed that even inconsistent pairing of a CS with sexual reward was able to elicit conditioned approach behaviour in male gerbils. In sum, even long CS-US intervals or inconsistent pairing of the CS with the US can result in sexual learning.

US Factors: Research has suggested that sexual learning can take place without permitting subjects to complete copulation (Hollis et al., 1989). Zamble et al. (1985) demonstrated conditioning in male rats that were permitted to intromit but not ejaculate. Second, they also found conditioning effects in male subjects when both intromission and ejaculation were precluded. Kippin and Pfaus (2001a,b) examined the copulatory components that comprised the US for the development of CEP. Male rats were allowed either multiple intromissions without ejaculation, single ejaculation, or multiple ejaculations with almond-scented females. Subsequently, copulatory preferences for an almond scented female or an unscented female were assessed. It was demonstrated that the development of CEP is dependent upon ejaculation with a scented female during the conditioning sessions. When male rats were not allowed to ejaculate during conditioning sessions, they did not demonstrate a preference. Moreover, the researchers demonstrated that the presence of the scented female following ejaculation is critical to the development of CEP in male rats. Moreover, they postulate that because the USs for CEP and USs for

conditioned sexual excitement are different, not all conditioning effects on sexual behaviour may involve the same US. Holloway and Domjan (1993) determined that completed copulation with a female quail was more effective than exposure to a female without copulation, but the latter also produced conditioned approach responding to the CS. Hilliard et al. (1998) devaluated the US by sexually satiating male quail. They found that consummatory responses (e.g. mount and cloacal contact) showed a stronger decline with sexual satiation, compared to appetitive responses (e.g. approach responses).

2.5.1.1. Aversive conditioning studies

Some studies have demonstrated that aversive conditioning can also influence sexual behaviour in animals. By the administration of lithium chloride (LiCl), copulation-illness associations can be induced. Reduced sexual motivation and longer intromission latencies are found when male rats and hamsters are injected with LiCl following copulation, or after exposure to estrous females (Ågmo, 2002; Peters, 1983; Johnston et al., 1978; Koch & Peters, 1987). Another study demonstrated that when domestic pony stallions are exposed to erection-contingent aversive conditioning with electric shocks, erection responses and attention to stimulus mares was suppressed (McDonnell et al., 1985). On the contrary, other research has shown that the pairing of a CS with a painful shock can induce copulation in noncopulating male rats (Crowley et al., 1973). The researchers conditioned sexually inactive male rats to associate tones with electric shock to the flanks. Eventually, the tones were presented when males were in the presence of an estrous female. The tones induced copulation with extreme regularity and the authors attributed this finding to an arousal augmentation by the anticipation of pain. Earlier research already suggested that the sexual drive of male rats seemed to be augmented by the nonspecific arousal reaction associated with the shock. Barfield and Sachs (1968) administered electric shocks to the back of sexually experienced males

that were in the presence of an estrous female. The administration of a shock, compared against a control condition in which no shock was delivered, could facilitate sexual activity. Other research by Caggiula and Eibergen (1969) with sexually naïve rats revealed that shocks induced copulatory behaviour toward estrous females in nearly four times as many animals as in a no-shock control condition. Shocks could also induce copulatory behaviour toward other male rats or toward stuffed toy animals. Interestingly, other behaviours like feeding or drinking were not influenced by tail shocks. It seemed therefore that the facilitative effect of pain-induced arousal is limited to certain behaviour like sexual activity that is associated with high levels of activation.

2.5.1.2. Reward system and dopamine receptor antagonism

Animal studies support involvement of the mesolimbic dopaminergic systems in sexual conditioning. West et al. (1992) trained one group of male rats to associate novel odours with a sexually receptive female, and one group with an unreceptive female and another group of rats received no training. They found that pairing odours with the presentation of sexually receptive females enhanced the responsiveness of NAc neurons to those odours. This was even more pronounced in those animals that were allowed to ejaculate during training than in animals that were not. Kippin et al. (2003) examined the pattern of neural activation in rats as revealed by Fos immunoreactivity (Fos-IR) after exposure to either the neutral odour of almond, which was paired previously with copulation with a receptive female, estrous odours, or no odour. It was demonstrated that estrous and sexually conditioned odours were processed by distinct neural pathways, which both included the NAc core. The authors postulate this structure has a unique role in processing sexual stimuli.

The effect of DA in the expression of conditioned level changing in rats in bilevel chambers has been investigated by Pfaus and Phillips (1991). In a bilevel box procedure, the number of level changes of a male rat in anticipation

of a female is thought to reflect sexual motivation. Male rats in bilevel chambers normally display an increase in level changing in anticipation of the arrival of a sexually receptive female (Pfaus et al., 1990), but administration of a D1 or D2 receptor antagonist produces a decrease in frequency of conditioned level changing in male rats. Also infusions of Haloperidol in the NAc, anteriodorsal striatum and mPOA were investigated and only infusions into the NAc and medial preoptic area decreased conditioned level changing. Furthermore, López and Ettenberg (2002) investigated the role of DA in mediating the positive value and behaviourally activating effects of a sexually conditioned cue. They conditioned male rats to associate two neutral olfactory cues with copulation and social isolation respectively. The rats' approach behaviour toward the scent was taken as an objective measure of its motivational value. Conditioned subjects were treated with different doses (0.0, 0.075, 0.15 or 0.30 mg/kg) of haloperidol 45 minutes prior testing their motivation to approach either the CS+ or CS− scents. Their data revealed that an olfactory cue associated with sexual reward can become a conditioned incentive that is capable of eliciting approach behaviour. Secondly, they found evidence for a dopaminergic role in mediating these motivational effects, as control subjects given vehicle injections took significantly less time to approach the CS+ than an unscented goalbox. This decrease in run latency was not observed in subjects within the 0.075 and 0.15 mg/kg haloperidol groups. These results support the notion that sexual reward is a powerful mediator of incentive formation and enhancement, and such associations are mediated by DA functioning.

2.5.2. Female studies

In contrast to the large number of male studies on sexual behaviour, the number of studies on conditioning of female sexual arousal and sexual behaviour are less abundant. To the best of our knowledge, no empirical

research on aversive conditioning of the sexual response of female animals has been conducted, but several studies have addressed appetitive conditioning.

Gutiérrez and Domjan (1997) have shown that when a visual stimulus is consistently preceding the arrival of a male quail, females approach and remain near the CS. Research has shown that CPP also develops in female rats and hamsters, although more robust preferences are seen in males (Oldenburger et al., 1992; Paredes & Alonso, 1997; Paredes & Vasquez, 1999; Arzate et al., 2011). Research suggests that CPP in female rats may be stronger associated with paced, than with nonpaced mating (Paredes & Alonso, 1997). Female paced mating behaviour is the pattern of approach and withdrawal during sexual encounters or the opportunity for the female to escape. In nonpaced mating environments the male controls the tempo of sexual interactions and the female cannot escape from the testing environment (Erskine, 1989). Research has shown that paced copulation induces greater DA release in NAc and striatum in female rats compared with nonpaced copulation (Mermelstein & Becker, 1995). This increase in DA does not depend on the amount of vaginocervical stimulation received from the male, but on the amount of paced vaginocervical stimulation (Becker et al., 2001). Pacing is the critical factor for sexual rewards in the female rat, and only in paced-mating studies the willingness of a female rat to initiate and engage in sexual interaction is reflected, whereas this is not the case in non-paced studies. A study by Pfaus and colleagues (Coria-Avila et al., 2005) demonstrated conditioned partner preference in female rats for males scented with an arbitrary odour that had been paired with paced copulation. This study demonstrated that also in female rats, neutral odours can acquire sexual incentive value and modulate partner preference when paired with the rewarding effects of paced copulation. Subsequent studies by Pfaus and colleagues (Coria-Avila et al., 2006) demonstrated that even though female rats have an unconditioned preference to copulate with males of the same strain, this preference can be switched

toward males of a different strain if that male is associated with the sexual reward induced by paced copulation.

Meerts and Clark (2009) tested the hypothesis that both vaginocervical stimulation and social interaction (placing a male rat into the arena with the experimental rat) can induce a CPP in female rats. Their study showed that female rats expressed a CPP for the context paired with nonpaced mating or artificial vaginocervical stimulation. These findings provide support for the notion that not only pacing or control per se is necessarily the rewarding element in copulation, but rather that vaginocervical stimulation is also an important aspect of the reinforcing effect of mating. Pfaus and colleagues (Parada et al., 2010) studied the ability of clitoral stimulation to induce CPP. They concluded that a form of stimulation is able to induce CPP, similar to the studies by Meerts and Clark (2009) that used vaginocervical stimulation. They also demonstrated that compared to no stimulation, clitoral stimulation induced Fos in hypothalamic and limbic structures, including the NAc. The authors suggest that clitoral stimulation induces a reward state in female rats. In a subsequent study by Pfaus and colleagues (Parada et al., 2011) it was investigated if clitoral stimulation could also induce a conditioned partner preference. In this study they paired a neutral odour with clitoral stimulation. Results suggest that clitoral stimulation of female rats indeed can induce partner preference. In a more recent study (Parada et al., 2013) it was demonstrated that sexual experience prior to conditioning presumably generates a US pre-exposure effect, which makes female rats less responsive to external clitoral stimulation alone. In this experiment female rats remained sexually naïve or received 1 or 5 copulatory sessions prior to conditioning. The female rats were able to pace the copulatory stimulation. Females that received 5 copulations did not develop a significant CPP to external clitoral stimulation, whereas females with either the no prior copulatory experience, or those who received 1 copulation, developed significant CPP to external clitoral stimulation. Thus,

external clitoral stimulation can be devalued as a reward by copulatory experiences.

Furthermore, two studies by Pfaus and colleagues (Coria-Avila et al., 2008a,b) examined the neurochemical basis of conditioned partner preference in female rats. The authors demonstrated that sexual reward in the form of paced copulation in female rats involves the activation of brain opioid systems. In their study, one group of female rats were conditioned to associate scented and unscented males with paced and nonpaced conditioning. The other group of females was conditioned to associate albino or pigmented males with paced or nonpaced copulation. Before each conditioning trail naloxone (an opioid antagonist) or saline was administered. The authors found that the naloxone-trained female rats showed no preference to copulate with either a pacing related or nonpaced related male rat. They concluded that opioids mediate the conditioned partner preference induced by paced copulation. In the second study, with a similar procedure as the first, they concluded that DA transmission is implicated in odour conditioning but is not necessary for the conditioning of strain cues of sexual reward. The administration of flupenthixol, a D1 and D2 receptor antagonist, disrupted odour conditioning but not strain conditioning. This suggests that in conditioned partner preference, the role DA plays depends on the type of stimuli to be learned. The authors suggest some cues may be more potent in terms of activating mesolimbic DA unconditionally. This could explain why it appears to be easier to condition strain cues to sexual reward. They speculate that it is possible that the necessary DA release to induce a significant increase of Fos in the NAc is simply not activated once strain cues have been conditioned. The authors suggest that it could be that strain cues alone produce more DA release than neutral olfactory cues before their association with sexual reward. This makes some associations more easy to learn and possibly less sensitive to disruption by DA antagonists (Coria-Avila et al., 2008a,b).

A more recent study by Arzate et al. (2011) allowed female rats to pace sexual interaction. This study was also designed to compare the rewarding properties of paced mating and morphine (opioids) injections. One group of females was allowed to paced mating before being placed in a non-preferred compartment. Later, they intraperitoneally received a morphine injection before being placed in the nonpreferred compartment and in alternate sessions they received a morphine injection before being placed in the preferred compartment. In the other group, treatments were reversed. Only females placed in the originally non-preferred compartment after paced mating changed their original preference. These results suggest that paced mating induces a positive reward of higher intensity than the intraperitoneally given morphine injection of 1 mg/kg.

Taken together, sexual behaviour can be modified by positive sexual and negative experiences in a wide range of taxa. Data from studies with male rats suggests that experience with ejaculation activates the reward system and sensitizes mesolimbic systems associated with incentive motivation. In female rats, the experience with paced copulation appears to activate the same reward and incentive systems. In quail species, a similar effect may underlie the copulatory reward in both males and females. To conclude, results from animal studies support the notion that sexual reward is a powerful mediator of incentive formation and enhancement, and such associations are mediated by DA functioning. Although in recent years a few very interesting studies have been published by Pfaus and co-workers, in animals the role of learning in sexual arousal and sexual behaviour has been studied less extensively in females than in males. Unfortunately, this is not only the case within animal studies, but also in human studies as the next sections will illustrate. Before discussing the research on conditioning of the human sexual response, we first briefly discuss used methods within this field of research.

2.6. Conditioning studies on human sexual response

In human research, the US is a sexually arousing stimulus rather than copulation with a receptive mate. As seen in animal studies, humans can show CRs to the CS, and secondly, humans are able to detect the nature of the contingency between CS and US. Evaluation of states of sexual arousal in human studies are usually measured by self-reports, rating tasks or questionnaires that ask subjects about their level of sexual arousal or other feelings. The CRs are measured by objective procedures such as physiological responding or behavioural recording (Lovibond & Shanks, 2002). The physiological components of sexual arousal include changes in genital response: erection in men and vaginal vasocongestion in women (Janssen et al., 2000; Laan et al., 1995a,b). Erectile responsiveness in men can be recorded by genital measurement devices on penile volume, circumference, and rigidity (Janssen et al., 2006). In laboratory use, penile strain gauges that measure circumference are most widely used. Female physiological sexual arousal can be measured by vaginal photoplethysmography. In this technique, a photometer detects increases in blood volume in the vaginal wall and yield vaginal pulse amplitude (VPA). In the following sections we first will discuss literature available on the classical conditioning of the sexual response in men, after which we will discuss the studies conducted in women. We will focus on the more recent literature, although we will refer to former studies, where they were of particular significance. Before discussing the studies on classical conditioning, we will first briefly consider studies on habituation of sexual arousal.

2.6.1. Male studies

2.6.1.1. Habituation of male sexual arousal

O'Donohue and colleagues demonstrated habituation of erectile responses with repeated exposure to the same erotic slides (O'Donohue & Geer, 1985) and to

erotic audiotapes (O'Donohue & Plaud, 1991). In contrast, a study by Smith and Over (1987) demonstrated no habituation in erectile responses or self-reported sexual arousal, while male subjects engaged in structured sexual fantasy over different trials. Since the subjects of this study had earlier participated in another study to employ strategies designed to maintain consistency in sexual arousal over trials, these results should be interpreted with caution. In a later study by Koukounas and Over (1993), sexual arousal habituated with repeated erotic stimulation through both fantasy as well as film material. They also found for both modes that when the repetitive stimulus was replaced by novel content, sexual arousal recovered. The authors pointed out that a change in absorption could have caused the habituation effects in the film condition, because attention to the film fragment had shifted from a participant perspective to a spectator perspective. In a subsequent study, Koukounas and Over (2001) addressed whether sexual arousal fails to habituate when participants maintain a constantly high level of absorption across repeated episodes of erotic stimulation. For this, they used two instructional sets that differed in their manipulation of absorption (i.e. emotion-based attention vs. stimulus-directed attention). The researchers found that under each instructional set, there was a reduction in sexual arousal during repeated erotic stimulation. After controlling for the changes in attentional focus, sexual arousal remained relatively stable over trials. Koukounas and Over suggested that sexual arousal is less likely to habituate if attentional focus remains constant during repeated erotic stimulation. Given the different methodologies used in the studies discussed above, there are indications that male sexual arousal is prone to habituation, but it seems that attentional processes such as the involvement in the erotic stimulus mediate habituation effects.

In a more recent study, Dawson et al. (2013) used erotic film clips with explicit sexual content to investigate habituation in both men and women. Subjects were presented with nine presentations of the same erotic clip,

followed by two presentations of different erotic clips and two presentations of the original erotic clip, while measuring genital responses by mercury-in-rubber strain gauges or vaginal photoplethysmography. Following each stimulus, participants rated their sexual and genital arousal as well as their level of attention during the stimulus using a nine-point scale. The researchers found that repeated exposure to the same erotic stimulus caused a reduction of genital responses in both men and women. Furthermore, subjective reports of sexual arousal showed a similar decline. Also neither sex showed dishabituated responses when re-exposed to the habituated stimulus. The researchers only found a sex difference in the initial response to sexual stimuli. Compared to women, men reported greater initial arousal to the habituation stimulus and their subjective sexual arousal followed a more marked decline with repeated exposure. Taken together, as the discussed studies have shown, male sexual arousal is prone to habituation.

2.6.1.2. Classical conditioning of the sexual response in human males

The most recent reviews on the conditioning of human sexual arousal have been provided by Akins (2004) and Hoffmann (2007). In an earlier review by O'Donohue and Plaud (1994), the authors concluded that the evidence for a relationship between classical conditioning and sexual behaviour was thin. Nevertheless, results from studies since then show that human studies may link more closely with animal studies with regard to the role of the nature of the CS or stimulus relevance in eliciting sexual arousal than perhaps thought (Akins, 2004).

As early as the work of Krafft-Ebing (1929), accidental pairing of an abnormal stimulus with sexual arousal or ejaculation is thought to be 'at base' of the development of sexual deviations. Interpretation of the results from earlier studies on sexual conditioning is complicated by methodological problems. Nevertheless, we will discuss some noteworthy studies. One of the

first studies on the conditioning of sexual arousal is that of Lovibond (1963). In this study it was demonstrated that the galvanic skin response can be conditioned in heterosexual males by using film presentation with graphic symbols as CS and pictures of nude females as US. Also, Rachman (1966) demonstrated sexual arousal in three adult male participants to a pair of black boots, using penile plethysmography. As US, a slide of an attractive nude woman was used. A conditioned response was defined as five successive penile responses to the CS. However, a minimum size of penile responding was not defined in advance. More important, the experiment did not rule out the possibility that it could have been the US itself that has led to sexual responding to the CS. To eliminate methodological criticism, Rachman and Hodgson (1968) replicated their study. In the experimental condition, the CS was contingently paired with the US. But this time, to control for the possibility of pseudo-conditioning, in the control condition the US was presented prior to the CS. The study was successful in reproducing the findings of the earlier study, but under a backward conditioning procedure no sexual responses to the boots were evoked. Subsequently, in a variant of the experiment by Lovibond (1963), McConaghy (1967, 1970) modified the film as used in the Lovibond experiment, by including pictures of male nudes, allowing for the study of classical conditioning in heterosexual as well as homosexual subjects. Film fragments of young adult women and men were shown alternately. Films of the women were preceded by photographs of a red circle, those of the men by photographs of a green triangle. Conditioned penile volume increases occurred for the CS paired with the US of the preferred sex and detumescence for the CS paired with the US of the nonpreferred sex. An important comment on this study made by Langevin and Martin (1975) was that the found detumescence for the CS paired with the US of the non-preferred sex, might have been due to natural detumescence following a period of arousal, and not to conditioned responses. Besides, as with the Rachman studies, no random control procedure

was utilized. Kantorowitz (1978) further examined the nature of association between the US and conditioned arousal induced by erotic slides in eight heterosexual male volunteers. During eight conditioning sessions, for each subject, three different slides of nude women were paired with the plateau, refractory, and resolution stages of masturbation. Stimuli paired with the plateau phase produced an increase in penile erection and stimuli paired with the refractory phase produced a decrease in erection. Not only may classical conditioning have been responsible for the increase in penile response to the stimuli paired with the plateau phase, the orgasm may have conditioned the sexual response in an operant manner as well (Dekker & Everaerd, 1989).

Inspired by the results of the experimental studies by Rachman and McConaghy and colleagues, theories arose about the aetiology of what was then called sexual deviant behaviour (McGuire et al., 1965). The application of behavioural techniques for the reorientation of sexual desires in an attempt to 'treat' these sexual behaviours increased exponentially during the following years. In most cases aversion techniques were applied in attempts to alter sexual behaviours (Solyom & Miller, 1965; McConaghy & Barr, 1973; McConaghy, 1975; Laws et al., 1978). Most, if not all of these studies lacked proper control procedures and often described single case studies. Nevertheless, these studies all suggested that sexual arousal can be classically conditioned.

Since then a number of well controlled studies have demonstrated that classical conditioning can augment sexual arousal in men. Two studies using nonclinical samples have shown convincing evidence for conditioning of male sexual arousal. In an experiment by Lalumière and Quinsey (1998) 10 male participants were exposed to a slide of a nude women and a highly arousing film clip of heterosexual sexual interactions. The other 10 participants were only exposed to a slide depicting a nude woman. Participants exposed to the slide plus the film clip showed increased penile responses to this slide of a nude woman, relative to other responses to other test stimuli (other slides of semi-

clothed women, two neutral slides that depicted trees and flowers). Participants exposed to only the slides depicting a nude woman, showed a reverse pattern: they showed a decrease in sexual arousal to the slide. The authors proposed that the finding of increased penile responses to the original slide with a nude woman, relative to the responses to other test stimuli (other slides of nude women) in the group that was also presented with the film clip, could be produced by inhibition to stimuli that were not associated with the US. The inhibition to the stimulus (slide of the nude woman) in the group that was not presented with the erotic film clip may have been the result of habituation, because the participants were exposed 11 times to the slide. The authors concluded that because the group difference was due to a relative increase in arousal to the stimulus of a nude woman when paired with an arousing sexual stimulus, and a relative decrease in arousal to the stimulus of a nude woman when presented alone, any attempts to increase sexual arousal using classical conditioning may be hindered by the male tendency to habituate to stimuli when presented repeatedly.

Plaud and Martini (1999) conditioned male sexual arousal in 9 subjects, using sexually explicit slides as US. As CS they used a slide of a penny jar. In the conditioning procedure, the CS was presented for 15 s, followed by the US for 30 s. The second procedure was identical to the first conditioning procedure, except that the US was presented before the CS. In the third procedure, the presentation of the CS and US was determined randomly. The results of this study indicated that subjects showed increases in penile tumescence from baseline in the first mentioned condition procedure, but not during the other control conditions.

A more recent study by Hoffmann et al. (2004) found sexual conditioning effects in men, but these effects only approached a conventional level of statistical significance. In their study both male and female subjects received subliminal or conscious presentations of a photograph of either a

sexually relevant or irrelevant CS, which was followed by an erotic film clip. Both men and women showed greater conditioned sexual responding when a subliminal CS was more sexually relevant (the abdomen of an individual of the opposite gender) than when it was less relevant (a gun). This supports the notion of stimulus relevance in humans. However, when consciously perceived CSs were used, gender differences came into view. Women showed conditioned arousal to the sexually irrelevant rather than the relevant CS, whereas men receiving conscious presentations of the CS showed more evidence of conditioned sexual arousal to the abdomen than to the gun.

In contrast to the study of Hoffmann et al., results from a fMRI study of Klucken et al. (2009) provide stronger evidence for conditioning in men than in women. Klucken and colleagues investigated neural activation during sexual conditioning. They presented a geometric shape (a rhomb) as CS+. This figure was followed by highly sexually arousing pictures (US). Another geometric figure (a square) served as CS− and was followed by neutral pictures. Arousal ratings were significantly higher for the CS+ than for the CS−. Second, greater activations in response to the CS+ were seen in reward related structures (e.g. OFC, VTA and ventral striatum) compared to the CS−. Furthermore, subjects who were aware of the contingency of the CS and US as compared to unaware subjects showed greater hemodynamic responses in the ventral striatum, medial OFC, occipital cortex, and VTA in response to the CS+ compared with the CS−. And also interesting, compared to women, men showed stronger conditioned activation in the amygdala, thalamus and occipital cortex. The researchers considered the results to be in line with other findings (Pfaus et al., 2001; Gutiérrez & Domjan, 1997), suggesting that men are more receptive to conditioning of sexual arousal than women.

More recent, Hoffmann et al. (2012) used a field study design to explore the conditioning of male sexual arousal in a real-world setting. The experiment was divided into a baseline and testing session in the laboratory and

intervening conditioning sessions in the field (e.g. participant or partner residence). They instructed seven heterosexual couples to include a novel, neutrally preferred scent as a CS+ during sexual interaction and another scent during non-sexual coupled-interaction like watching a movie together. Hereto, females were given two white cotton tops, two aroma fans and vials with geranium and basil essential oils. In a control group seven couples used both scents during non-sexual interaction. Over a 2-week period both experimental and control couples had three sexual interactions. Furthermore, experimental couples had three non-sexual interactions, while controls had six non-sexual interactions. The female partner was responsible for orchestrating 'conditioning' and was instructed to alternate activities (sexual and non-sexual) and to spread them out for more than 12 h apart. During the baseline session and the testing session in the lab, men placed electromechanical strain gauges on their penis and positioned an oxygen cannula for odour presentation. Subsequently, they were exposed to presentations of basil and geranium odours, while listening to relaxing music accompanied by media player visuals on a computer monitor. This procedure was repeated with two different odours, but now participants were exposed to short fragments of a non-sexual film and a sexual film. The final testing session was similar to the baseline session except that odorants were presented in a different order. In the experimental group, an increased genital responding to the CS+ was observed relative to the control group. In addition, males in the experimental group also showed a trend for decreased preference for the CS− odour. Somewhat contrary to what the authors expected, the genital CRs were not stronger than those obtained during laboratory-based sexual conditioning. Nonetheless, participants retained the CR for at least several days (since participants, although instructed did not return straight to the laboratory for testing after completion of the conditioning phase). This study does show a longer-retention of conditioned sexual responses than has typically documented in humans. This

study also provides evidence for evaluative sexual conditioning in men, as they found an increase in preference for the partner-paired odour in the control group and the sexually paired odour in the experimental group.

In summary, studies providing empirical evidence for conditioning in human male sexual arousal are scarce. As a result of procedural problems and confounds, the interpretation of results from former studies within this field is limited. Nevertheless, latter studies contribute to the growing evidence that classical conditioning procedures can condition the male sexual response. The next section will focus on empirical evidence for operant conditioning in male sexual arousal.

2.6.1.3. Operant conditioning of the sexual response in human males

There are several studies on the regulation of genital responses as a function of instrumental contingencies. Rosen and colleagues (Rosen, 1973; Rosen et al., 1975) conducted two studies to investigate the possible modification of penile tumescence by instrumental conditioning. In the first study (Rosen, 1973) as a result of contingent feedback highly significant suppression of tumescence relative to the flaccid state was obtained. In order to elicit penile tumescence, a series of erotic tape-recordings were presented. A red light was presented to male volunteers whenever their erection exceeded a criterion increase, and in this way they learned to significantly inhibit tumescence over the course of three experimental sessions. In their second study, Rosen and colleagues gave subjects in the experimental group analogue visual feedback and monetary rewards for increases in penile tumescence in the absence of external erotic stimuli. Rosen and co-workers concluded that instrumental conditioning is effective in modifying sexual arousal. However, they did not test for sexual fantasizing.

Inspired by the results obtained in classical conditioning procedures on the treatment of particular sexual behaviours, several case studies have reported

pairing sexual arousal produced by masturbation with heterosexual stimuli in the treatment of homosexuality or undesired sexual behaviour. But in order to 'treat' these forms of sexual behaviour, many case studies describe aversive conditioning (Bancroft, 1969, 1970; Feldman, 1966; Freeman & Meyer, 1975; Herman et al., 1974a,b; Josiassen et al., 1980; Gold & Neufeld, 1965; Quinn et al., 1970; Beech et al., 1971; Rosen & Kopel, 1977). For example, MacCulloch and Feldman (1967a,b) used aversion therapy in homosexual subjects. Subjects could avoid a shock after seeing a male picture by turning off the slide by pressing a switch. Thereby, the introduction of female slides was made contiguous with the removal of the male slides. Over half of these subjects reported a change in sexual orientation during this experiment, but no follow-up was conducted. In their second study a follow-up study was conducted. Eventually a substantial number of participants were said to have shown a change in sexual orientation that was still present at one-year follow-up. However, the reliability of self-report as a treatment outcome measure may be questioned. Also other types of clinical conditioning techniques like that of orgasmic reconditioning (guided fantasy in masturbation) or covert sensitization were applied in order to shape sexual behaviour (Abel et al., 1970; Abel & Blanchard, 1974; Alford et al., 1987; Jackson, 1969; MacCulloch et al., 1971; Brownell et al., 1977; Schaefer & Colgan, 1977). The evidence in support of these procedures used alone is mixed (Conrad & Wincze, 1976; Lande, 1980; VanDeventer & Laws, 1978) and again, most of these studies concern uncontrolled (case) studies (Conrad & Wincze, 1976; Keller & Goldstein, 1978). The study of Marquis (1970) is a good example of an orgasmic reconditioning study. Subjects with 'perversions' were instructed to masturbate to the point of orgasm (using a preferred fantasy), at which time the subject was instructed to switch to more 'appropriate' fantasies. Almost all subjects were reported to be cured, or to be improved. Because of the lack of objective measures, again these results cannot be interpreted straightforwardly. Although

there has been some empirical evidence that learning plays a role in the development of partner preferences, and partner preferences appear to be influenced by experiences both early in life and in adulthood, the general assumption is that the orientation-preference is at least in part determined by sexual imprinting (Pfaus et al., 2001; Woodson, 2002).

Even though no firm conclusions can be drawn from most of those uncontrolled operant conditioning studies because possible evidence does not go beyond the case study level, combined with the results from classical conditioning studies, they do point to the conclusion that conditioning procedures can affect the human sexual response. Although it is unlikely that conditioning procedures can alter sexual orientation, conditioning can influence the (motivational) valence of an incentive. Although it may be expected that the same effects should occur in women, literature on this topic is scarce. First we will discuss the studies of habituation of female sexual arousal and continue with studies on classical conditioning.

2.6.2. Female studies

2.6.2.1. Habituation of female sexual arousal

Parallel to the habituation effects that O'Donohue and Geer (1985) established for men, Meuwissen and Over (1990) demonstrated decreased levels of genital and subjective sexual arousal in women, after repeated exposure to erotic fantasy as well as erotic film fragments. There was recovery of arousal to the original response level, when a new erotic stimulus, i.e. a new fantasy or film fragment was introduced. A later study to assess the occurrence of habituation in female subjects after repeated stimulus exposure could however not draw reliable conclusions considering habituation of female sexual arousal (Laan & Everaerd, 1995a,b). After 21 trials of sexual stimulus presentation, female subjects continued to show both genital and subjective arousal. In a more

recent study from our laboratory a habituation design with 'hot vs. cool' attentional strategies was used to investigate the regulation of sexual arousal (Both et al., 2011b). Manipulation of a hot and a cool attentional focus was done by instructing participants to either imagine that they were engaged in the sexual activities depicted in the erotic film stimulus and to focus on their physiological and emotional reactions that they felt during film viewing (hot focus), or to realize that they were "just looking at a film" and to focus on characteristics of the physical setting of the erotic film (cool focus). Under the hot focus stronger sexual feelings were observed than under the cool focus. Furthermore, habituation and novelty effects were found: during repeated erotic stimulation genital responses and sexual feelings diminished and with the introduction of a novel stimulation they increased. Interestingly, the hot attentional focus did not preclude habituation of sexual arousal. As earlier mentioned, the study by Dawson, Suschinsky and Lalumière (2013) showed that men and women showed statistically similar patterns of genital responding to repeated stimulation. Women showed diminution of both genital responses and subjective reports of sexual arousal. The authors concluded that there is a possibility that sexual responses of men and women are similarly malleable and equally subject to learning processes. Despite, there seems to be some evidence for female sexual arousal to habituate, more research is needed on this topic and it is too early to draw firm conclusions.

2.6.2.2. Conditioning studies on female sexual arousal

To the best of our knowledge, in contrast to the numerous comparable reports on men, only one case of a conditioning procedure in an attempt to alter female homosexuality was published. Blitch and Haynes (1972) described a case study of treating a female homosexual by systematic desensitization and manipulation of masturbation fantasies. The participant was instructed to make image switches when her masturbation image was initially homosexual. After the

termination of the therapy, an increase in heterosexual images of masturbation were said to have taken place. A follow-up study stated that no overt homosexual behaviour had occurred since the therapy. Since this study was solely based on self-report measures, no further conclusions can be made.

The first controlled conditioning study on female sexual arousal study was that of Letourneau and O'Donohue (1997). The authors tried to condition subjective sexual arousal and vaginal blood flow. They used film fragments showing heterosexual oral or coital sex and as a neutral stimulus they used amber light. A significant conditioning effect was not detected. They attributed these results to an ineffective US. Opposing to this study, Hoffmann et al. (2004) found statistically significant evidence for conditioned genital arousal in women, as discussed earlier. When the CS was presented subliminally, men and women showed more conditioned arousal to the sexually relevant CS than the sexually irrelevant CS (gun). When the CS was consciously perceived, women showed stronger conditioned arousal to the sexually irrelevant CS compared with the relevant one. It was suggested the latter finding was due to an increase in automatic nervous system responding such that women showed greater general arousal to the nonsexually relevant CS than to the sexually relevant CS. This pleads in favour of the assumption that there is an independent role for automatic processing in human sexual response mechanisms. Their findings are consistent with Öhman and Soares (1994) in demonstrating that associative learning in humans can occur without awareness of the CS-US contingency. In a comparable study, in our lab unconscious classical conditioning of sexual arousal in women was investigated (Both et al., 2008a). Clitoral vibrostimulation served as US and two subliminally presented erotic pictures served as CS+ or CS−. With a forced choice recognition task, conscious perception was tested. Results from this task revealed that participants were not able to perceive the CSs during masked presentation. Thus, evidence was found for a conditioned genital response following repeated pairing of masked erotic pictures with

genital vibrostimulation and these findings add to the increasing evidence for associative emotional learning without awareness of the CS-US contingency (Öhman & Soares, 1993, 1994, 1998; Wong et al., 2004; Hoffmann et al., 2004).

A later study in our lab (Both et al., 2008b) was the first that provided evidence for a conditioned genital response to an initially neutral CS in human females. Again a differential conditioning procedure was used with genital vibrostimulation as US. Two neutral pictures of cartoon male faces served as the CS stimuli. A conditioned genital arousal response to the neutral picture that was paired with genital vibrostimulation was found. Secondly, a relation was observed between the strength of the conditioned genital arousal response and sexual functioning and sexual arousability as measured by questionnaires. It was suggested that a person who is more easily sexually conditionable may associate several stimuli with rewarding sexual experience and subsequently, this may result in sexual arousal and desire when confronted with these cues. In a following study, a few changes were made compared to the earlier study (Both et al., 2011a). Skin conductance level (SCL) and subjective affective value was measured as well as genital and subjective sexual arousal in response to the CSs. Again they observed a conditioned sexual arousal response, as genital arousal was higher in response to the CS+ than in response to the CS−. Also, a marginally significant difference was found for affective value ratings between the CS+ and CS−, indicating a more positive evaluation of the CS that was followed by genital vibrostimulation.

In addition, a differential conditioning study on aversive conditioning was conducted (Both et al., 2008b). In this study evidence was found for attenuation of female genital response through aversive conditioning. This time two erotic pictures served as CS and a painful stimulus at the wrist as US. After conditioning VPA was lower in response to the CS+. Moreover, the CS+ was rated more negative compared to the CS−. Although this is the only study on aversive sexual conditioning in women to date, these results support the view

that in women, as a result of classical conditioning, aversive sexual experiences may result in decreased sexual arousal.

The results obtained by Hoffmann and colleagues, and Both and colleagues, oppose the study by Letourneau and O'Donohue (1997) in which no evidence was found for female conditioned genital response. As stated before, Letourneau and O'Donohue suggested that the failure to demonstrate conditioned sexual arousal may have been because of an ineffective US. However, in the studies in our laboratory genital responses to the US were also weak, suggesting that a strong responding to the US is not a prerequisite to demonstrate conditioned genital arousal in women. More interesting is the fact that Letourneau and O'Donohue used a coloured light as CS, whereas in the studies by Hoffmann et al. (2004) and our studies (Both et al., 2008a,b, 2011a) more sexually relevant CSs were used (i.e. a picture of a male abdomen and a picture of a male face). Possibly the conditioning of female sexual arousal may be facilitated by the use of those more sexually relevant CSs. Also interesting to note is the finding in our studies that conditioned genital response as well as conditioned affective value did not clearly diminish during the extinction phase. This suggests resistance to extinction of conditioned sexual responses. However, since the number of extinction trials in these studies was small, firm conclusions regarding the presence or absence of extinction of conditioned responses cannot be made.

Concluding, despite the limited amount of research, there is growing support that female sexual arousal can be conditioned as well. But evidence is scarce and efforts to replicate these studies are encouraged.

2.7. Discussion

Sexual behaviour of animals can be modified by positive sexual and negative experiences in a wide range of taxa. Arbitrary CSs such as auditory, visual and olfactory stimuli appear to be effective CSs in both male and female animals,

but even more intriguing, greater CS-US similarity appears to elicit different CRs, at least in male animals. In contrast to results obtained with arbitrary CSs, conditioned responses toward sexually relevant CSs might be highly resistant to extinction, at least in male quail. Therefore, CS-US similarity seems to be an important factor in conditioning (Rescorla & Furrow, 1977; Krause et al., 2003). Furthermore, it seems that even long CS-US intervals or inconsistent pairing of the CS with the US can result in sexual learning in animals. Moreover, this sexual learning can take place without permitting subjects to complete copulation (Hollis et al., 1989; Zamble et al., 1985; Holloway & Domjan, 1993). But Kippin and Pfaus (2001a,b) demonstrated that the development of CEP in male rats is dependent upon ejaculation. And because the USs for CEP and USs for conditioned sexual excitement are different, not all conditioning effects on sexual behaviour may involve the same US. Furthermore, results from animal studies support the notion that a state of sexual reward, by for instance ejaculation in male rats or paced mating or vaginocervical/clitoral stimulation in female rats, is a powerful mediator of incentive formation and enhancement, and such associations are mediated by DA functioning.

Research on human sexual conditioning has lagged substantially behind that of animal sexual functioning. Nevertheless, studies in humans have demonstrated that classical conditioning can augment or diminish subjective and genital sexual arousal in both male and female subjects. Human studies may link more closely with animal studies with regard to the role of the nature of the CS or stimulus relevance in eliciting sexual arousal than perhaps thought (Akins, 2004). We will discuss the most prominent overlaps.

The mechanism of preparedness may cause some events or stimuli to become more easily associated than others. First, the results of both the study by Hoffmann and colleagues and Letourneau and O'Donohue support the notion of stimulus relevance in humans as well as animals. As we have

mentioned before, Hoffmann et al. (2004) found no clear evidence of conditioning in human males as their effects in men only approached a conventional level of statistical significance, but both men and women showed greater sexual responding when a subliminal CS was more sexually relevant than when it was less relevant. The lack of a significant conditioning effect in the Letourneau and O'Donohue (1997) study in women may have been due to their use of a sexually irrelevant CS. It is possible that the conditioning of female sexual arousal may be facilitated by the use of sexually more relevant CSs.

The results of the study by Hoffmann and colleagues do not entirely support this hypothesis of preparedness. In their study they used a picture of an abdomen of an individual of the opposite gender as 'relevant' CS. Interestingly, experimental eye-tracking studies on viewing patterns of sexual stimuli have shown that when men and women are presented with the same sexual stimuli, they do not view them in the same manner (Rupp & Wallen, 2008). Both men and women spent less time looking at a male body look zone than would be expected based on the average proportion of picture area it occupied. This may mean that heterosexual female participants are not interested in the male bodies, unless genitalia are depicted. A study by Lykins et al. (2006) revealed that nude male bodies are relevant for heterosexual women. When the genitals are included in the male body look zone, women do preferentially view nude male bodies. Possibly, this reflects a female preference for looking at male genitalia. It could be that a picture of an abdomen of an individual of the opposite gender, as used by Hoffmann and co-workers, was not the evident relevant CS after all, at least for women. It would be interesting to use explicit pictures of genitals of the opposite sex as CSs in future sexual conditioning studies in women. Future studies should therefore focus on sexually relevant and sexually irrelevant CSs. It would be very interesting to further investigate if some aspects of sexual stimulus processing in humans do indeed involve

preferential rapid learning to certain classes of innately 'prepared' stimuli (Seligman, 1971; Öhman, 1986; Öhman & Mineka, 2001). But as mentioned before, the preparedness theory is not incontrovertible. Therefore, the rapid learning to certain classes of stimuli can also be explained by experience with certain stimuli (Davey, 1995). For instance, Masataka (1993) demonstrated that experiences with certain stimuli that are not snakes (e.g., live insects) can differentially sensitize a fear of snakes in laboratory-bred monkeys, suggesting that experiences with selected non snake stimuli can influence subsequent fearful reactions to snakes in some direct or indirect way. In a similar way, possible rapid learning to sexual relevant stimuli could also be explained by past experiences and the cognitive ability to infer that there are important functional differences between CSs. Nevertheless, conditioned responses to sexually relevant CSs seem highly resistant to extinction in animals and findings in humans point to the same direction. Results from the sexual conditioning studies by Both and colleagues (Both et al., 2008a,b, 2011a,b) showed that conditioned subjective affect does not extinguish significantly during the extinction phase. This suggests a resistance to extinction. This finding is in line with evaluative conditioning research (Vansteenwegen et al., 2006). Acquired subjective likes and dislikes are seemingly quite resistant to extinction. Such a resistance to extinction may have important clinical implications. When conditioned valence is indeed relatively resistant to extinction, in treatment of hypersexuality and paraphilia a combination of extinction therapy with counter conditioning, thus learning new opposite responses, would be more effective than extinction therapy alone. But more research should be conducted within this field to be conclusive. Second, future studies could also investigate this possible resistance to extinction with an implicit measurement, like an approach and avoidance task (Chen & Bargh, 1999; Wiers et al., 2011). Self-report measures assess mental processes or representations that are consciously accessible. In contrast, implicit measures assess automatic processes that often

operate outside awareness. As subjective measures are prone to socially desirable bias, it would be interesting for future studies to include an implicit measure.

Moreover, in animals, sexual conditioning experiments indicate involvement of limbic reward circuitry, including the NAc. To date, only one fMRI study on human sexual conditioning has been reported, using erotic pictures as US (Klucken et al., 2009). Nevertheless, also conditioned activation was seen in reward structures. Since vibrostimulation has proven to be an effective US in women, it would be interesting to conduct a similar study using a tactile US instead of a visual US. A tactile US may yield stronger conditioning effects.

To conclude, how do stimuli acquire sexually arousing properties? The ability of sexual behaviour, especially orgasm and ejaculation, to increase the concentration of DA in the NAc is considered to be crucial for the acquisition of new sexual learning in humans (Berke & Hymann, 2000; Di Chiara, 1999; Robinson & Berridge, 2003). Stimuli that resemble some innate sexual US more closely, are thought to become more easily associated with a state of sexual reward. But by the means of powerful mediating DA functioning, also sexual irrelevant stimuli can become associated with sexual reward. The brain stores an internal representation of experiential sexual events, and DA neurons mediate associative learning and expectations based on previous experience with a stimulus. Furthermore, repeated exposure to a sexual rewarding stimulus causes strengthening of stimulus-response and stimulus-reward associations, and sensitizes the mesolimbic pathways (Di Chiara, 1999). Subsequently, sexual rewards evoke an increased DA release into the NAc. Repetitive sexual rewards result in releasing Delta FosB in the NAc. Delta FosB may cause sensitization to sexual rewards (Nestler, 2001). In addition, since orexin is thought to facilitate glutamate-mediated responses, and to be necessary for glutamate-dependent long-term potentiation in VTA DA neurons, it is suggested that the

altered glutamate sensitivity by exposure to sexual rewards strengthens the neural pathways that link memories of the sexual stimulus event and related cues with high reward (Esch & Stefano, 2004; Hyman et al., 2006; Chen et al., 2010; Wise & Morales, 2010; Kelz et al., 1999; Aston-Jones et al., 2010; Fields et al., 2007).

Bancroft and Janssen (2000) proposed a theoretical model of dual control of sexual arousal and associated behaviours in which the net expression of sexual behaviour is based on the influence of excitatory and inhibitory mechanisms in the brain. As in Gray's biopsychological theory about behavioural activation and inhibition systems (Gray, 1982) this model stresses the adaptive nature of both excitatory and inhibitory processes. The dual model by Bancroft en Janssen can be described as a theory of approach and avoidance and the associated concepts of reward and punishment. The authors assume that sexual inhibition and excitation are both adaptive, serving a number of biological functions. Although they suggest that learning possibly plays a role in determining individual variabilities in response tendencies, they assume that individual variation in sexual inhibition and excitation is a stable trait, which is possibly genetically determined (Janssen & Bancrtoft, 2007). Individuals with an unusually high propensity for excitation or a low propensity for inhibition are more likely to engage in high-risk sexual behaviour. In contrast, individuals with a low propensity for sexual excitation or a high propensity for sexual inhibition are more likely to experience problems with impairment of sexual response (Bancroft et al., 2009). The model of dual control of sexual arousal allows the sexual response systems to be flexible. Flexibility in sexual responding possibly results in a sexual system that can exist under a variety of optimal and even unoptimal internal and external conditions. As Pfaus et al. (2001) state it "Flexibility in responding gives different species an enormous amount of chance to recombine in different ways, yielding a higher degree of diversity." Nevertheless, learning processes constrain the individual's attention to sets of

stimuli and patterns of behaviour that result in the proximal goal of sexual reward (in other words: stimuli that are known to "work"). Such stimuli are likely to differ from person to person based on every individual's sexual history, and this also accounts for the related behaviours. This means that how excited or how aroused an individual becomes by a certain stimulus is determined by the counterbalancing of experience and conditioning on one hand and instinctual responses to unconditionally arousing stimuli (both internal secretions and external cues) on the other. Pfaus (2009) presented an overview of neurochemical and neuroanatomical systems involved in sexual inhibition and excitation. During sexual inhibition the opioid, endocannabinoid, and serotonin systems are activated. In contrast, the DA systems, melanocortin, oxytocin, and norepinephrine systems are activated during sexual excitation. The core of sexual excitation pathway includes the mPOA and its outputs to the VTA. Brain pathways for sexual inhibition involve the activation of inhibitory opioid, endocannabinoid, and serotonergic feedback to various levels of the excitatory pathway. The inhibitory pathway is activated by sexual stimulation that reaches critical thresholds for sexual reward, sedation, and satiety. Since the excitatory pathway is stimulated both hormonally and conditionally by the expectancy of sexual rewards, external incentive stimuli can act as occasion setting for the excitatory system.

Despite the observed convergence between animal studies and human studies, there are many topics that need further exploration in the study of human sexual conditioning. Results from a study by Both and colleagues (Both et al., 2005) support the view that DA is involved in the energetic aspects of appetitive sexual behaviour, at least in men. And a more recent fMRI-study from our lab (Oei et al., 2012) provides compelling evidence for a mediating role of DA in processing of subconscious perceived sexual stimuli. In healthy young men, levodopa significantly enhanced the activation in the NAc and dorsal anterior cingulate cortex in response to subliminal sexual stimuli,

whereas haloperidol (a DA blocker) decreased activations in those areas. This first evidence for pharmacological modulation of implicit sexual reward processing, points at the possibility for DA to affect sexual motivation at its earliest onset, that is, outside awareness. But as these results only apply for men, further studies are warranted to investigate the role of DA in female sexual behaviour. Moreover, as enhancing effects of testosterone administration on neural activity related to appetitive goal attainment in mesolimbic incentive processing circuits has been demonstrated (Hermans et al., 2010), these findings may have implications for the understanding of why relatively more men are prone to develop hypersexuality or paraphilia (Krueger & Kaplan, 2001; Kafka & Hennen, 2003).

Moreover, no human research has been done on the role of dopamine in sexual reward learning, while facilitation as well as impairment of sexual reward learning is relevant in the context of treatment of hypo- and hypersexual desire disorder. Future studies on sexual behaviour also need to investigate how DA affects the incentive reward of neutral stimuli paired with rewarding ones. Presumably, the combination of individual differences in DA sensitivity in combination with frequent exposure to sexual cues and reinforcement processes might explain the initiation of aberrant sexual desires. The use of an implicit task in future studies would also be interesting because, as stated before, according to the incentive-salience hypothesis the dopaminergic pathway of the reward system attributes incentive salience to representations of stimuli or events that were associated with appetitive reward. As Berridge (1996) underlines that both wanting and liking can exist without subjective awareness, reward processing and learning can occur without conscious experience (Damasio & Carvalho, 2013). On the contrast of implicit emotion as a corresponding label for unconscious affective reactions, explicit emotion refers to the person's conscious awareness of an emotion, state or feeling (Berridge & Winkielman, 2003). Although emotions can occur unconscious and

people are not able to report their emotional reaction at the moment it is caused, this emotional reaction can be visible in their behaviour, physiological responses or subjective impressions of an-affect laden event. In this way, sexual stimuli can activate bodily responses (and implicit memory) before conscious appraisal. For this reason it would be very interesting to study dopaminergic effects on implicit sexual reward learning in human beings. Although there is evidence for enhanced tendencies to approach sexual stimuli following DA increased activation (Both et al., 2005) no study to date investigated whether DA-dependent increased activations by for instance conditioned sexual stimuli are related to increases in approach behaviour.

What can be said about possible gender differences in sexual conditioning at the moment? As little research has been done within the field of conditioning of the sexual response in humans, and most of this conducted research is limited to one of the sexes, exploration of possible sex differences is not straightforward. Nevertheless, we will discuss the most remarkable possible sex differences. First, as the research with male rats by Pfaus and co-workers has shown, sexual reward is a powerful mediator of incentive formation and enhancement, and those associations are mediated by DA functioning. But as the studies by Pfaus (Coria-Avila et al., 2008a,b) have shown, the administration of a D1 and D2 receptor antagonist disrupted odour conditioning but not strain conditioning in female rats. This suggests that in conditioned partner preference, the role DA plays in female rats depends on the type of stimuli to be learned. In humans, little research has looked into the role of DA in sexual motivation and sexual reward processing. Moreover, no studies to date have addressed the role DA plays in mediating associative sexual learning in both men and women. Therefore, future research is warranted to explore possible gender differences. Second, as studies in rats have shown, tones paired with electric shocks in an aversive conditioning procedure, can induce copulation in noncopulating male rats. And this finding was attributed to an arousal

augmentation by the anticipation of pain. Unfortunately, to the best of our knowledge, to date no empirical research on aversive sexual conditioning in female animals has been conducted. Therefore, we do not know whether such a finding would be observed in female rats. But in women, we found attenuation of sexual response by aversive conditioning (Both et al., 2008b). Research on pain-related fear on sexual arousal in women revealed that the threat of a painful shock to the wrist impeded genital arousal and positive affect, whereas it amplified negative affect (Brauer et al., 2007). Therefore, one might conclude that expected pain is likely to reduce sexual arousal in women. Furthermore, it would be interesting to investigate if electric shocks during a conditioning procedure could facilitate sexual arousal in human males. Third, results from the fMRI study by Klucken et al. (2009) revealed stronger conditioned activation in the amygdala, thalamus and occipital cortex in men compared to women. The researchers considered the results to be in line with other findings (Pfaus et al., 2001; Gutiérrez & Domjan, 1997), and subsequently suggested that men are more receptive to conditioning of sexual arousal than women. But since there is so little literature within this field it is too early to suggest such a difference in conditionability between men and women. Gender differences in the number of DA neurons are influenced by several factors, including sex chromosome complement (Lombardo et al., 2012), the presence of the sry gene (Dewing et al., 2006) and as discussed in more detail before, gonadal hormones. Therefore it is conceivable that that gender differences in conditionability do exist. But since research on conditionability and related neuromodulatory systems in humans is in its infancy, we can only speculate if similar processes can be proven to account for any gender differences that may be found in future human studies. We can only conclude that not only more research is needed in bridging the gap between animal studies and human studies, but also future replication studies in both men and women are needed before we can say anything about possible gender differences in sexual conditioning.

As has been shown, much empirical evidence from both animal and human studies fits the assumptions of the incentive motivation theory. According to the model, individual differences in sensitivity for certain sexual stimuli can predict which incentives are preferred, and which are not. Interestingly, research has demonstrated that sexual arousal is preference-specific in men, whereas women have a nonspecific pattern of sexual arousal (Chivers et al., 2004). Heterosexual men are more aroused by female than by male sexual stimuli and homosexual men show the opposite pattern. In contrast, the genital responses of women are only modestly related to their preferred category. Thereby, genital arousal in women is not per se accompanied by subjective desire or arousal (Laan & Everaerd, 1995a,b; Laan et al., 1995a,b). In relation to the incentive motivation theory, that states that sexual motivation is the result of the interplay of a sensitive internal sexual system with external motivational stimuli, this implies that for homosexual men for example, a picture of an attractive nude man may be innately sexually competent, but for heterosexual men this is likely not the case. As mentioned, research does confirm such a mechanism, at least for men. But with respect to women, genital responses are not very informative about sexual preference. Only subjective arousal reflects their preference. Notwithstanding the importance of incentives, individuals are not simply passive until triggered by the matching preferred external incentives or the cues associated with them. Cognitive processes like conscious awareness, goals and social restrictions can influence this process. Although men and women do not differ with respect to basic sexual learning, it is speculated that women are more sensitive to variations in social and cultural factors (i.e., exhibit more erotic plasticity") compared to men (Baumeister, 2000; Toates, 2009). In women, a sexual stimulus tends to trigger a wider range of cognitions as compared to men (Laan & Janssen, 2007). Therefore it is suggested that women's sexual motivation and arousal might be more strongly controlled by cognitive factors, whereas men's

sexual motivation tends to be more strongly controlled by stimulus factors. The above mentioned findings make clear that the theory of incentive motivation captures in a simplified form only some of the processes that underlie human sexual motivation and behaviour (Toates, 2009). Further insights require looking more closely at the pathway of information between stimulus and response and considering how the processes captured by the original incentive motivation model are embedded within other (higher-level) processes.

As DA is not the only neurotransmitter involved in the sexual system, future studies should also look at the serotonin system. Pharmacological manipulations of the central serotonin neurotransmitter system alter functioning of the hypothalamicpituitary- adrenal (HPA) axis. Such HPA changes play important roles in behavioural modulation and may contribute to the alteration of sexual and social behaviour (Aubert et al., 2012). For example, serotonin has sexual side effects such as decreased sexual desire (Meston & Frohlich, 2000), and serotonin reuptake inhibitors seem to have efficacy in treating hypersexuality (Bradford, 2001). Flibanserin, a postsynaptic 5-HT1A agonist/5-HT2A antagonist, has been shown to increase sexual desire and reduce sexual distress in women with Hypoactive Sexual Desire Disorder (HSDD) (Kennedy, 2010; Thorp et al., 2012). Therefore, it would be worthwhile to examine how serotonin possibly inhibits the reward system activity during processing of sexual stimuli, and study the role of serotonin in associative sexual reward learning in general.

In addition to DA, opioids are also differentially involved in conditioned and unconditioned sexual behaviours (Holloway, 2012). The brain opioid system is involved in sexual reward in the form of ejaculation in male rats, or paced copulation in female rats (Pfaus & Gorzalka, 1987; Pfaus et al., 2013) and is believed to be the main modulator of sexual reward (Ågmo & Berenfeld, 1990; Coria- Avila et al., 2008a,b). In the distinction made by Berridge, 'wanting' has been characterized as the value of incentive motivation

held by a stimulus without any hedonic component, and mediated by DA functioning. On contrast, 'liking' encompasses the hedonic aspect of a stimulus presentation, the positive sensory component that accompanies reward delivery (Berridge, 2004) and is thought to be mediated by the opioid system. Research has revealed two different domains in which endogenous opioids, present in separate and distinct brain regions, are involved (van Ree et al., 2000). One is related to incentive motivation, in which opioid systems in the VTA and the mesolimbic DA system are involved, and may relate to sexual motivation. The other is the performance of certain behaviours involving endogenous opioids, like sexual performance. In general, opioids and opioid drugs are found to have an inhibitory role in both male and female sexual behaviour (Pfaus & Gorzalka, 1987; Holloway, 2012). Research has demonstrated that opioid actions in the VTA potentiate mesolimbic DA activation, whereas opioid actions in the mPOA inhibit sexual behaviour in rodents (van Furth et al., 1995; Holloway, 2012; van Ree et al., 2000). Administration of opioid antagonists (e.g. naloxone) in sexual conditioning experiments has shown to disrupt the incentive motivation for and/or hedonic value of a CS predicting sexual opportunity or of the sexual stimulus itself (Holloway, 2012). Moreover, opioids have been implicated in mediating 'wanting' through their activity in the amygdala. The central nucleus of the amygdala is involved in the translation of learning into motivation. Mahler et al. (2009) demonstrated that opioid stimulation of the central nucleus of the amygdala in rats magnified and focused learned incentive salience onto a specific reward cue (CS). This motivation enhancement made the CS more attractive, resulting in more appetitive and consummatory behaviours. The authors concluded that opioid neurotransmission in the central nucleus of the amygdala is involved in the process of making one reward cue more "wanted" than others. Unfortunately, to date no studies on the role of opioids in human sexual conditioning have been conducted (for an overview on the role of opioids in learned sexual behaviour in animal see Holloway, 2012).

Seen the importance of the opioid system in mediating sexual reward, future research is needed.

As the aversion conditioning studies that attempted to alter certain sexual behaviours have demonstrated, there has been some empirical evidence that learning possibly can play a role in the shaping of partner preferences via conditioning. But since no robust or long lasting changes in sexual orientation in humans have been reported, the general assumption to date is that the orientation-preference is, at least in part, determined by sexual imprinting (Woodson, 2002; Swaab, 2008). In animal models, there are documented effects of conditioning on sexual arousal, approach behaviour, sexual performance and strength of sexual preference toward opposite-sex targets, but also no robust and long-lasting demonstrations of learning in the organization of same-sex preferences among males could be found (Nash & Domjan, 1991; Pfaus et al., 2001). Although some studies with rats have shown that as a result of repeated cohabitation under the effects of a D2-type receptor agonist, rewarding associations with same-sex individuals can facilitate socio-sexual partner preference in male rats (Coria-Avila, 2012; Cibrian-Llanderal et al., 2012; Triana-Del Rio et al., 2011), this result could also be interpreted as not solely a homosexual preference, but rather as a same sex social preference over receptive females. Moreover, the observed preference could also be a preference for a familiar social partner over a novel unfamiliar one, rather than a preference for male rats over receptive females.

In comparison to the substantial amount of research on anxiety and aversive conditioning in animals and humans, and despite its relevance for extinction-based treatments, little attention has been devoted to the phenomenon of renewal in appetitive conditioning. In treatments using cue exposure, for example in addiction disorders or anxiety disorders, people are exposed to conditioned stimuli while preventing their learned response, to extinguish cue-activated responses. However, many people relapse after being

'cured'. An important question is how to explain the return of responses following extinction procedures. Although CS-alone presentations may extinguish conditioned responses, the extinction procedure does not erase the originally learned association. It seems that this original association is retained (Bouton & Moody, 2004). This retention of the original association has been shown by renewal. This suggests that extinction is especially dependent on context. To the best of our knowledge human studies on extinction and renewal in the sexual domain are completely lacking. Translating the renewal phenomenon to the sexual domain, as noted before, a patient who is craving for internet-sex may be successfully extinguished by cue exposure therapy in a specific context, but may experience strong craving upon changing context such as sitting behind a different computer. Therefore, because of their clinical relevance, future studies on renewal of conditioned sexual responses should be given attention. As conditioning of subjective sexual arousal and genital arousal in humans can occur without awareness of the CS-US contingency, another prominent research question for future research within this field would be which brain systems are involved in conscious and unconscious sexual reward learning and in the regulation of sexual emotion. The investigation of brain responses during conscious and unconscious sexual learning will lead to new basic knowledge, and will underscore the importance of implicit processes. The level of detail that has been achieved in humans pales in comparison to the animal studies on neural circuits involved in conditioned learning. The amygdala contributes to both appetitive and aversive states. Studies in primates have shown that appetitive and aversive signals are processed by distinct neuronal populations of cells in the lateral/basal amygdale (LeDoux, 2012). LeDoux's (1996, 2012) work on the role of the amygdala is of great influence on thinking about unconscious activation of emotions. LeDoux discovered parallel transmission to the amygdala from the thalamus and the cortex (1996, 2000). The thalamo-amygdala projections appear to be involved in the

processing of the affective significance of relatively simple sensory features and this is the fast, direct route. The thalamo-corticoamygdala projections are necessary when more complex aspects of stimuli are processed. As LeDoux has shown, relative simple sensory processing by subcortical areas can provide the requisite inputs to structures such as the amygdale, bypassing or short-circuiting cortical areas (LeDoux, 1996). For example, humans can recognize certain emotions by the eyes alone and do not need to process the face as a whole (Whalen et al., 2004). Research has shown that this processing occurs subcortically (see LeDoux, 2012). Although there is an ongoing debate in visual attention research about what information is exactly transmitted from the thalamus to the amygdala (i.e. likely no geometric information due to the receptive-field properties of the superior colliculus, but more likely only gross luminance changes; Redgrave et al., 2008), it is possible that this same principle accounts for the processing of sexual stimuli too. In a world flooded with sexual stimuli, subconscious processes, as Oei et al. (2012) have shown, might play a role in compulsive reward-seeking behaviours such as hypersexuality. Cognitive behavioural therapy more and more incorporates treatments that target implicit processes. For example, attentional bias training in abstinent alcoholic patients (Schoenmakers et al., 2010), cognitive bias modification in depression (Holmes et al., 2009), and retraining automatic action tendencies in hazardous alcohol drinkers (Wiers et al., 2010) have been shown to be promising treatment possibilities. These interventions can also be promising for the treatment of sexual arousal disorders. In the case of hypersexuality, retraining automatic action tendencies with an approach/avoidance-task or attentional bias training could be fruitful interventions, whereas in the case of decreased sexual arousal not only retraining automatic action tendencies, but also cognitive bias modification using mental imagery can be a possible form of clinical intervention.

There are many topics that need future investigation in the study of sexual associative learning and the brain. The described list of possible future research directions is only meant to point out some of the obvious examples that need further attention. Taken together, the suggested future research may strongly influence ideas about disordered sexual motivation, and will hopefully contribute to the development of treatments for hypoactive and hyperactive sexual desire.

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Chapter 3

The Effect of a Dopamine Antagonist on Conditioning of Sexual Arousal in Women

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Abstract

Dopamine (DA) plays a key role in reward-seeking behaviours. Accumulating evidence from animal and human studies suggests that human sexual reward learning may also depend on DA transmission. However, research on the role of dopamine (DA) in human sexual reward learning is completely lacking. To investigate whether DA antagonism attenuates classical conditioning of sexual response in humans, healthy women were randomly allocated to one of two treatment conditions: Haloperidol (n=29) or Placebo (n=29). A differential conditioning paradigm was applied with genital vibrostimulation as unconditional stimulus (US) and neutral pictures as conditional stimuli (CSs). Genital arousal was assessed and ratings of affective value and subjective sexual arousal were obtained. Haloperidol administration affected unconditional genital responding. However, no significant effects of medication were found for conditioned responding. No firm conclusions can be drawn about whether female sexual reward learning implicates DA transmission, since the results do not lend themselves to unambiguous interpretation.

3.1. Introduction

The dopaminergic reward system has been implicated to be involved in the acquisition and expression of learned appetitive behaviours (Dominguez & Hull, 2005; Fields et al., 2007; Schultz, 2007; Richard et al., 2012), and abnormality in this system has been shown to play a role in the aetiology and pathophysiology of various disorders, including substance use disorders and (behavioural) addictions (De Jong et al., 2015; Dunlop & Nemeroff, 2007; Root et al., 2015). Many theories of human sexual behaviour assume that sexual stimuli can obtain arousing properties through associative (classical/Pavlovian) learning processes (Brom et al., 2014a; Pfaus, Kippin & Centeno, 2001; Toates, 2009). Therefore, the onset of disorders in sexual motivation such as Female Sexual Interest/Arousal Disorder (Diagnostic and Statistical Manual of Mental Disorders [DSM-5] American Psychiatric Association, 2013) or hypersexuality, may be explained from a classical conditioning and incentive motivation perspective (Brom et al., 2014a; Laan & Both, 2008; Singer & Toates, 1987). However, despite the substantial amount of research that suggests that mesolimbic dopamine (DA) neurotransmission plays an important role in aversive learning (Zweifel et al., 2011) as well as reward learning (Berridge, 2007; Berridge & Robinson, 1998, 2003; Brom et al., 2014a; Di Chiara, 1995; Kringelbach & Berridge, 2009) to date, no human research has been conducted on the role of DA in human sexual reward learning, while facilitation as well as impairment thereof is relevant in the context of treatment of sexual motivation disorders.

Stimuli that can promote motivation are called incentive stimuli (Bindra, 1974; Singer & Toates, 1987). Their motivational valence can be unconditional or conditional as a result of associative leaning (Di Chiara, 1995). A previously neutral stimulus (NS) that predicts reward (i.e. unconditional stimulus; US) can acquire motivational properties, becoming an attractive and desirable incentive stimulus (i.e. conditional stimulus; CS). As a result of

repeated association of the NS with the US, the NS may eventually trigger similar responses as the US (i.e. the conditioned response; CR) (Pavlov, 1927). However, it is important to mention that the NS does not always have to trigger the exact same response as the US does (Fanselow et al., 1994), and therefore the CR may not always equal the unconditioned response (UR). Subsequent repeated presentations of a CS without the US will result in a loss of conditioned responding (i.e. extinction), as the CS no longer predicts the appetitive US (Delamater, 2004). Several studies have demonstrated conditioned sexual arousal responses in animals and humans (Both et al., 2011; Brom et al., 2014a, b, c; Pfaus, Kippin & Centeno, 2001).

Rewards like food, drugs and sex, have the ability to stimulate mesolimbic DA neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), and increased extracellular concentrations of mesolimbic DA are implicated in responding for conditioned reinforcers (Berridge, 2007; Georgiadis & Kringelbach, 2012; Pierce & Kumaresan, 2006; Richard et al., 2012). A recent fMRI-study (Oei et al., 2012) provides compelling evidence for a mediating role of DA in processing of subconscious perceived sexual stimuli. In healthy young men, Levodopa (a DA agonist) enhanced the activation in the NAc and dorsal anterior cingulate cortex in response to subliminal sexual stimuli, whereas Haloperidol decreased activations in those areas. Both et al. (2005) demonstrated a relation between dopaminergic activity and motor preparation in response to sexual stimuli. Moreover, substantial evidence suggests that mesolimbic DA plays a critical role in the incentive and acquisition aspect of reward (Berridge, 2007; Schultz, 2002; Wise, 2002). The incentive salience theory describes mechanisms by which DA transmission in the NAc transforms the neural representations of conditioned stimuli, converting an event or environmental stimulus from a neutral representation into an attractive and 'wanted' incentive (Berridge, 2007; Flagel et al., 2010). Research has shown that DA agonists or DA uptake

inhibitors, such as D-amphetamine or methylphenidate increase conditioned responding in rats (Beninger et al., 1980; Cummins et al., 2014; Taylor & Robbins, 1984) and humans (Kassubek, Abler & Pinkhardt, 2011), whereas DA antagonists decrease conditioned responding in rats (Banasikowski et al., 2010; Ranaldi & Beninger, 1993; Wolterink et al., 1993). For instance, in rats, haloperidol selectively attenuates conditioned-cue induced sexual motivation (Coria-Avila, 2008; Lopez & Ettenberg, 2002). In humans, dopaminergic influences on reward learning were observed in studies by Pessiglione et al. (2006) and Pleger et al. (2009), in which participants were administered a single dose of haloperidol or levodopa preceding an instrumental learning task and a reward decision-making task respectively. Haloperidol attenuated and levodopa enhanced learning effects. However, in contrast, Pizzagalli et al. (2008) and Santesso et al. (2011) demonstrated that a single dose of the DA agonist pramipexole impaired the acquisition of reward-related behaviour in healthy participants. This blunted reward learning was explained by the assumption that low doses of pramipexole may influence reward via a paradoxical effect related to activation of the presynaptic DA autoreceptor, resulting in a blockade of phasic DA release and a blunted response to rewarding stimuli (Riba et al, 2008). As the mixed results make clear, the role of phasic dopamine (DA) signalling in incentive learning in humans remains largely unknown. Therefore, in the present study, making use of a double-blind, parallel-conditions, placebo controlled design, it was investigated whether DA antagonism attenuates classical conditioning of sexual response in women. It was expected that administration of the DA antagonist haloperidol would decrease the magnitude of the conditioned sexual response.

3.2. Method

3.2.1. Participants

A total of 58 healthy sexually active women from the general population were recruited by means of advertisements, and were randomly allocated to two treatment conditions: Placebo n=29, and Haloperidol n=29. The inclusion criteria were: age between 18 – 45 years and a heterosexual orientation, no pregnancy or breastfeeding, no current (or history of) sexual complaints as determined by the Female Sexual Function Index (FSFI; Rosen et al., 2000; Ter Kuile, Brauer & Laan, 2006) or psychiatric problems as determined by the MINI International Neuropsychiatric Interview (MINI; Sheehan et al, 1998); no history of sexual abuse; no medical illness (or medical history) indicating a risk in using haloperidol (e.g., cardiac illness, depression, thyroid disorders, glaucoma); no use of medication affecting sexual response; and no current or recent use (<12 weeks before participation) of psycho-pharmacological medication, psychotropic drugs, or medication that might interfere with haloperidol (e.g., cannabis or cocaine). Participants were paid €50 for their participation and written informed consent was obtained from all participants. The study was approved by the Medical Ethics Committee and carried out according to the standards of the Declaration of Helsinki (Declaration of Helsinki, 2000).

3.2.2. Medication

Participants received a single dose of haloperidol (3 mg, the mean time of maximal plasma levels, (T_{max})=3–6 h, half-time=14–36 h; Liem-Moolenaar et al, 2010), or placebo (microcrystalline cellulose), hidden in identical gelatine capsules to ensure that both participants and experimenters could not identify the drugs. Following dosing, participants rested for 3h to allow drug absorption. This timing was based upon a studies in healthy volunteers that

showed 60–70% D2 receptor occupancy and maximal plasma concentrations 3h after haloperidol administration (Darby et al., 1995; Nordstrom et al, 1992), and on research that showed haloperidol effects 1h after dosing on cognitive tests making use of a reliable Central Nervous System (CNS) measurement battery (Liem-Moolenaar et al., 2010). Moreover, previous studies from our lab (Oei et al., 2012) demonstrated decreased activations in brain reward structures 4h after oral ingestion of 3mg haloperidol.

3.2.3. Conditioning Procedure

The experimental design involved differential conditioning with one stimulus (the CS+) being followed by genital vibrostimulation (US) during the acquisition phase, whereas the other stimulus (CS-) was never followed by genital vibrostimulation. For a schematic overview of the procedure see Figure 1. In the preconditioning phase, participants saw four nonreinforced presentations of the CS+ and four presentations of the CS-, for 11s each. Subsequently, in the acquisition phase the CS+ and CS- were presented 8 times each and after 10s the CS+ was always followed by the US for 2s. In the extinction phase the CS+ and CS- were presented 6 times each, and now the CS+ was no longer followed by the US. All phases were presented without interruption. There were two random CS orders for each phase (that was counterbalanced across participants); with the restriction of only two successive presentations of each CS. During the whole procedure inter-trial intervals (ITIs) were 20, 25, or 30s. The order of the length of the ITI was random, with the restriction of only two similar successive lengths.

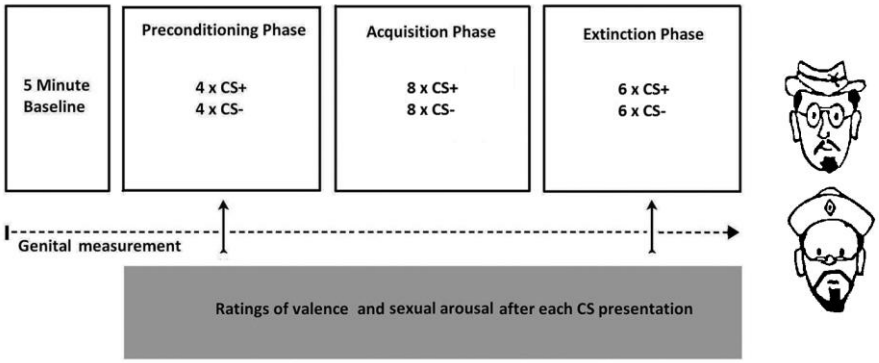

Figure 1. Schematic representation of the conditioning procedure and extinction phase, with on the right the used stimuli that served as CSs.

3.2.4. Stimulus Materials

CS

Two similar neutral pictures of pictorial male faces (Both et al., 2011; Brom et al., 2014b) served as CSs. The CSs were shown in the middle of a computer monitor, approximately 1.5 m in front of the participant. The size of the presented pictures was 14 X 21 cm. Assignment of the pictures as CS+ and CS- was counterbalanced across participants and conditions. A computer program timed the administration of the CS and US stimuli.

Genital Vibrostimulation (US)

A small hands-off vibrator (2 cm diameter) (Laan & van Lunsen, 2002) was placed on the clitoris using a lycra panties. All participants were instructed to position the vibrator as *most sexually stimulating*.

3.2.5. Genital Arousal

Vaginal photoplethysmography assessed vaginal pulse amplitude (VPA) (Laan et al., 1995). VPA is a reliable measure specific to sexual arousal (Laan, Everaerd & Evers, 1995; Suschinsky, Lalumière & Chivers, 2009). The photoplethysmograph is a menstrual tampon-sized device containing an orange-red light source and a photocell. The light source illuminates the capillary bed of the vaginal wall and the blood circulation within it. Depth of the probe and orientation of the light emitting diode were controlled by a device (a 6- X 2-cm plate) attached to the cable within 5 cm of the light sensor. The photoplethysmograph was disinfected at the medical centre by means of a plasma sterilization procedure between uses. Plasma sterilization is a highly effective method for the complete removal of all organic (and certain in-organic) material. Genital response was measured continuously during resting baseline, preconditioning, acquisition, and extinction phases.

3.2.6. Subjective Ratings

Ratings of affective value, and sexual arousal were collected during the preconditioning- and extinction phases. Participants were first asked to rate, after each CS presentation, the affective value of the CSs by answering the question "*What kind of feeling does this picture evoke in you*?" The question could be answered on a seven-point Likert scale on a keyboard that varied from very negative to very positive. Then, subjective sexual arousal was rated by answering the question "*How sexually arousing is this picture to you?*" The question could be answered on a seven-point scale that varied from not sexually arousing at all to very sexually arousing. The questions were presented at the monitor 1s following the end of picture presentation.

3.2.7. The Female Sexual Function Index (FSFI)

Women's sexual functioning was assessed by the FSFI (Rosen, et al., 2000; Ter Kuile, Brauer & Laan, 2006), consisting of six subscales: desire (two items; range 1–5), arousal (four items; range 0–5), lubrication (four items; range 0–5), orgasm (three items; range 0–5), satisfaction (three items; range 0–5), and pain (three items; range 0–5). A higher score indicates better sexual functioning. The FSFI has good internal reliability and is able to differentiate between clinical samples and functional controls (Rosen, et al., 2000; Ter Kuile, Brauer & Laan, 2006).

3.2.8. Procedure

A female who was a trained experimenter tested each participant individually. Women were not tested during menstruation. Before entering the experimental session participants were instructed about the genital device and vibrostimulation and informed consent was obtained. Participants received a capsule (placebo or haloperidol) 3h before the experimental conditioning procedure to ensure the occurrence of peak-plasma concentrations of the drugs during the experiment (Darby et al., 1995; Nordstrom et al., 1992; Liem-Moolenaar et al, 2010). After ingestion of the capsule, participants filled out questionnaires (e.g., FSFI). They were allowed to read during the waiting period. Exactly 3 h after ingesting the capsule, the experimental procedure started. Participants privately inserted the vaginal device and placed the vibrator. Further instructions were given through instructions on screen. Three presentations of vibrostimulation of 2s each allowed the participant to position the vibrator in the way it was most sexually arousing. It was emphasized that after final placement the position of the vibrator should not be changed during the experiment. A 5-minute resting period followed, during which a neutral film was played and baseline measurements of genital response were collected during the last 2 minutes. Subsequently, the conditioning procedure followed.

After the experiment finished, participants themselves removed the genital devices privately. Next, an debriefing interview was administered in which participants were asked about their sentiments with regard to the experimental procedure, the use of the genital device, and their evaluation of the genital vibrostimulation. Finally, participants were thanked and paid for their participation and advised to refrain from alcohol and drug use the next 24h.

3.2.9. Data Reduction, Scoring and Analysis

A software program (VSRRP98) was used to analyse the genital data. After artefact removal, mean VPA level during the 2-minute resting baseline period was calculated. Genital responses to the CSs were scored in three latency windows: during 4-8, 9-12 and 13-16s following CS onset, respectively FIR (first interval response), SIR (second interval response) and TIR (third interval response). These time intervals are based on previous data (Both et al., 2011; Brom et al., 2014b) showing that vaginal blood engorgement is a relatively slow physiological response. For FIR, SIR and TIR, change scores were calculated for each CS presentation by subtracting mean genital resting baseline from genital response following CS presentation. For genital responses and subjective ratings, effects were tested with repeated measures univariate analysis of variance procedures (General Linear Model in SPSS), with Stimulus and Trial as within-subject factors and Condition as between subjects factor. The Greenhouse–Geisser correction was applied to adjust for violation of the sphericity assumption in testing repeated measures effects. The preconditioning, acquisition, and extinction phases were analysed separately. Effect sizes are reported as proportion of partial variance (η_p^2) (Cohen, 1988). In addition, the strength of the unconditioned and conditioned genital response was determined. The magnitude of the unconditioned response (UR) was determined by calculating the percentage of preconditioning VPA score (mean

VPA in response to the CS+ plus vibration during the acquisition phase / mean VPA in response to the CS+ during the preconditioning phase * 100). The magnitude of the conditioned response (CR) was determined by calculating the percentage of the mean VPA in response to vibration (VPA in response to the CS+ during the first extinction trial / mean VPA in response to the CS+ plus vibration during the acquisition phase * 100).

		Placebo (*n*= 29)		Haloperidol (*n*= 29)		
		M	**SD**	**M**	**SD**	***p***
Age (years)		22.24	3.14	20.31	1.71	<.01*
Sexual Functioning (FSFI- score)		28.63	5.97	28.81	5.63	.90
Prior Experience Vibrostimulation		3.00	1.28	2.24	1.12	.02*
Pleasantness US		2.24	0.64	2.28	0.65	.84
US Perceived as Sexually Arousing		2.62	0.78	2.48	0.63	.46
Declared Sexual Arousal		2.03	0.73	2.14	0.69	.58
		Frequency		**Frequency**		
Use of Contraceptives	**No use or non-hormonal**	5		3		.72
	Hormonal	24		26		

Table 1. Participant characteristics. Descriptive subject variables for each condition. Notes: Women's sexual functioning was assessed by the Female Sexual Function Index (FSFI; Rosen et al., 2000; Ter Kuile et al., 2006). Questions from debriefing, Scales: Prior experience vibrostimulation: 1 (never) – 5 (very often); Pleasantness US: 1 (not pleasant at all) - 5 (very pleasant); US perceived as sexually arousing: 1 (not sexually arousing at all) – 5 (very sexually arousing); Declared sexual arousal: 1 (not sexually aroused at all) – 5 (very sexually aroused); * $p < .05$.

3.3. Results

The participants in the two conditions appeared to differ in age and in prior experience with genital vibrostimulation, see Table 1 Participant characteristics. Genital data from one participant (from the Haloperidol condition) were discarded as outlier since measures from this participant were under 3 SD from the mean (although inclusion of this participant did not change results). There was no relation between the medication the participants had received and the percentage that correctly guessed what they had received (Pearson Chi Square=2.75, p=.25), suggesting that blinding was succesful. Most participants reported no side effects (n=37). Among the 21 participants who did report side effects, the most commonly reported ones were fatigue, sleepiness, and dizziness. Participants in de Haloperidol condition reported more side effects as compared to participants in the Placebo condition (Fisher's exact test=6.05, p=.03). The most frequent reported side effect was dizziness.

3.3.1. Genital Sexual Arousal

Preconditioning Phase. Analyses were conducted to verify equal levels of VPA in response to the CS+ and CS- during the preconditioning phase. For all latency windows (FIR, SIR and TIR), no difference in VPA following presentation of the CS+ and CS- was found, all ps> .23. On TIR a significant Stimulus X Condition interaction effect was found, $F(1, 55)= 6.74$, $p= .01$, $\eta_p^2 = .11$. As can be seen in Figure 2, participants in the Haloperidol condition demonstrated higher VPA responses towards the CS-, whereas women in the Placebo condition had higher VPA in response to the CS+ in the preconditioning phase.

Acquisition Phase. VPA in response to the vibrostimulation during the acquisition phase was determined in order to verify whether the

vibrostimulation served as a sexually arousing US. Genital responses in the second and third latency windows (SIR, TIR) were considered as unconditioned responses. Figure 2 summarizes VPA TIR to CS+ and CS- across trials for both conditions separately. In line with previous studies (Both et al., 2008, 2011; Brom et al., 2014b), the 2 (Stimulus) X 8 (Trial) X 2 (Condition) mixed factors ANOVA of VPA revealed only a significant main effect of Stimulus on TIR, FIR p= .28, SIR, p=.11, TIR, $F(1, 54)$= 5.65, p= .02, η_p^2 = .10, meaning the CS+ plus vibrostimulation elicited higher levels of VPA. On FIR and SIR there were no significant Stimulus X Condition interaction effects, FIR p= .51, SIR p= .38, or Stimulus X Trial X Condition interaction effects, FIR p= .86; SIR p= .29. On TIR there was a trend for a Stimulus X Condition interaction, $F(1, 54)$= 3.16, p=.08, η_p^2 = .06, and for a Stimulus X Trial X Condition interaction, $F(5, 250)$= 2.19, p=.06, η_p^2 = .04, were seen. Inspection of Figure 2 suggests the Placebo condition demonstrated greater differential responding in the second half of the acquisition phase compared to the Haloperidol condition. Additional analysis of the first half of the acquisition trials (trials 1-4 of acquisition phase) yielded a significant Stimulus X Trial X Condition interaction on TIR $F(3, 143)$= 3.87, p< .02, η_p^2 = .07, and analysis of the second half of the acquisition phase (trials 5-8) yielded a significant Stimulus X Condition interaction on TIR, $F(1, 55)$= 4.65, p< .04, η_p^2 = .08. Because conditions differed in age, with the Placebo condition being significantly older than the Haloperidol condition (see Table 1), and in prior experience with vibrostimulation, and in the difference in VPA towards CS+/CS- during the preconditioning phase, additional analyses were conducted with those variables as covariates. On TIR again a significant main effect of Stimulus was seen, $F(1, 51)$= 5.23, p< .03, η_p^2 = .10, and a significant Stimulus X Condition interaction

effect, $F(1, 51) = 6.32$, $p < .02$, $\eta_p^2 = .11$. Also a trend for a Stimulus X Age interaction, $p < .09$, was seen. Concluding, the vibrostimulation resulted in a genital arousal response in both conditions, and results from the additional analysis showed that administration of a DA antagonist decreased the magnitude of differential responding towards the CS+ plus vibrostimulation and CS- in the second half of the acquisition phase.

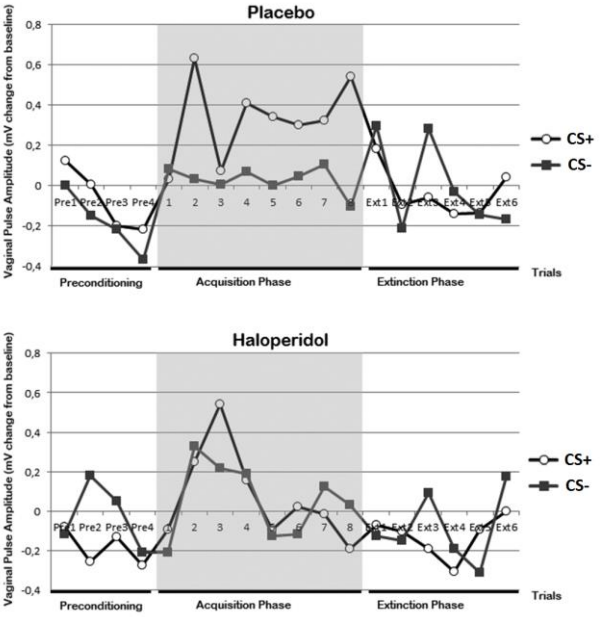

Figure 2. Mean vaginal pulse amplitude (VPA) change scores (with standard error bars) for the conditions Placebo and Haloperidol, during the third interval response window (TIR) following CS+ and CS- during the preconditioning phase, acquisition phase and extinction phase. Note that during the acquisition phase, the response represents responding to the CS+ plus the US[1].

[1] Given that animal studies on conditioned sexual response have revealed interactions between sex steroids and DA in the control of sexual behaviour (see Brom et al., 2014a) and have revealed influences of estrous cycle phase on conditioning and extinction (Milad et al., 2006), additional analyses were conducted, controlled for women during their early follicular phase (i.e., early cycle) and during the late follicular phase (i.e., midcycle). However, those analyses revealed no additional differences between conditions.

Extinction Phase. Analysis of the first extinction trial revealed no conditioned responding, FIR p= .47, SIR p= .32, TIR p= .64, with no differences therein between the conditions, FIR p= .93; SIR, p= .99; TIR p= .65. The 2 (Stimulus) X 6 (Trial) X 2 (Condition) mixed factors ANOVA of all extinction trials, also did not reveal conditioned responding, all ps> .19, and no Stimulus X Trial X Condition interaction, all ps> .53. However, a less stringent method, namely analysis of only the response towards the CS+ on the last preconditioning trial and on the first extinction trial revealed a difference in conditioned responding between the Placebo and Haloperidol condition, as reflected by a significant Trial X Condition interaction on SIR, $F(1, 54)= 4.89$, $p< .03$, =.08, and a trend for a Trial X Condition interaction on TIR $F(1, 54)= 3.81$, $p<.06$, = .07. Additional analyses were conducted with the variables Age, Prior Experience Vibrostimulation, and difference in VPA towards CS+/CS- during the preconditioning phase as covariates. Analysis of the first extinction trial again revealed no conditioned responding on FIR and SIR and no differences therein between conditions, all ps>. 28. On TIR a trend of Stimulus was seen, $F(1, 49)= 3.51$, $p< .07$, $\eta_p^2 = .07$, but a non-significant Stimulus X Condition interaction effect, p= .11. Additionally, the 2 (Stimulus) X 6 (Trial) X 2 (Condition) mixed factors ANOVA of all extinction trials now revealed conditioned responding on SIR, $F(1, 49)= 5.25$, $p< .03$, $\eta_p^2 = .10$, but no differences therein between conditions, p= .23. On TIR no conditioned responding was seen, again with no differences therein between conditions, all ps>. 10. The analysis of only responses towards the CS+ on the last preconditioning trial and on the first extinction trial revealed no difference in conditioned responding between the conditions, all ps>. 10. To conclude, administration of Haloperidol did not decrease the magnitude of the conditioned sexual response compared to the Placebo condition.

3.3.2. Subjective Measures

Preconditioning phase. The 2 (Stimulus) X 4 (Trial) X 2 (Condition) mixed factors ANOVA to verify equal levels of responding to the CSs revealed no difference in responding following presentation of the CS+ and CS- on affective value and subjective sexual arousal, or between conditions, all *p*s > .25.

Extinction Phase.

Subjective Affect. As can be seen in Figure 3, contrary to the expectations, for subjective affect there was no robust increase of differential responding towards CS+ and CS- after the acquisition phase. Analysis of the first extinction trial revealed no significant Stimulus X Condition interaction effect, $p = .98$, and no main effect of Stimulus, $p = .14$. Analysis of ratings of affective value during the preconditioning phase (Mean trial 1–4) and the first extinction trial, revealed no Stimulus X Condition interaction, $p = .83$, nor a Stimulus X Trial X Condition interaction, $p = .70$. The 2 (Stimulus) X 6 (Trial) X 2 (Condition) mixed factors ANOVA of all extinction trials revealed no differences between conditions, as reflected by non-significant Stimulus X Condition and Stimulus X Trial X Condition interactions, all *p*s> .64. A trend of Stimulus was seen, $F(1, 48) = 2.97$, $p = .09$, $\eta_p^2 = .06$. Analysis with Age and Prior Experience Vibrostimulation as covariates, showed a similar pattern of results.

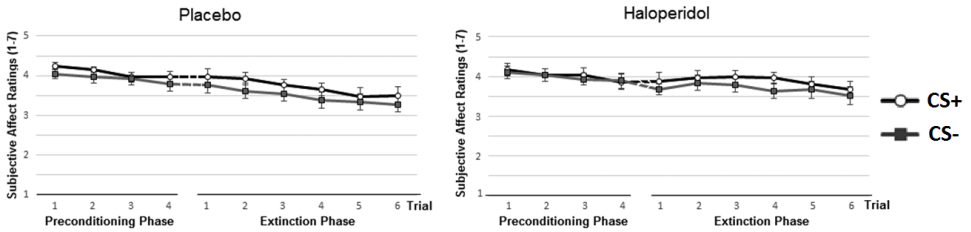

Figure 3. Subjective affect ratings (with standard error bars) following the CS+ and CS- during the preconditioning phase and extinction phase in the two conditions Placebo (left) and Haloperidol (right).

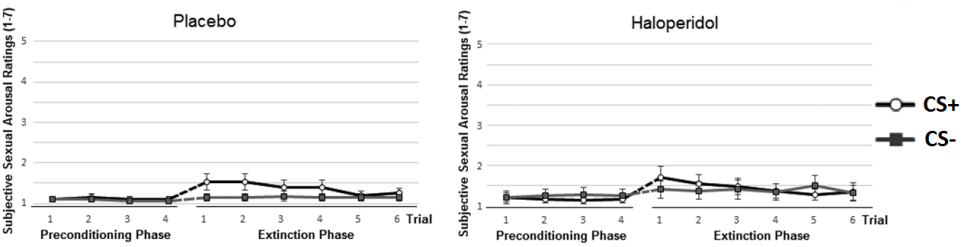

Figure 4. Ratings (with standard error bars) of subjective sexual arousal following the CS+ and CS- during the preconditioning phase and extinction phase in the two conditions Placebo (left) and Haloperidol (right).

Subjective Sexual Arousal. Figure 4 shows increased ratings of subjective sexual arousal towards the CS+ on the first trials of the extinction phase, indicating conditioned response, in both conditions. Analysis of ratings of subjective sexual arousal during the preconditioning phase (Mean trial 1–4) and the first extinction trial, revealed no significant Stimulus X Condition interaction effect, $p = .48$, or Stimulus X Trial X Condition interaction effect, $p = .91$. However, a significant main effect was seen of Stimulus, $F(1, 54) = 9.71$, $p < .01$, $\eta_p^2 = .15$, indicating conditioned responding. Analysis of the first extinction trial yielded no significant interaction of Stimulus X Condition, $p = .76$. Again a main effect of Stimulus was found, $F(1, 55) = 7.06$, $p = .01$, $\eta_p^2 = .11$. The 2 (Stimulus) X 6

(Trial) X 2 (Condition) mixed factors ANOVA of all extinction trials, revealed no significant interaction effects of Stimulus X Condition or Stimulus X Trial X Condition, both *p*s> .39. However, there was a significant Stimulus X Trial interaction effect, $F(3, 148) = 2.76$, $p = .04$, $\eta_p^2 = .06$, indicating extinction of conditioned responding. Analyses with Age and Prior Experience Vibrostimulation as covariates showed a similar pattern as reported above. To conclude, the modulation of dopaminergic tone with Haloperidol did not decrease the magnitude of conditioned subjective affect or sexual arousal as compared to the Placebo condition.

3.3.3. Magnitude of the Conditioned (CR) Genital Response

Compared to the unconditioned genital response, the magnitude of the CR during the first extinction trial was 116,8% and 98%, respectively, for VPA SIR and TIR for the Placebo condition. For the DA condition this was 72,2% and 51,1%. No significant differences were seen between conditions therein, all *p*s> .28.

3.4. Discussion

To investigate whether phasic DA signalling is a prerequisite in sexual reward learning in humans, dopaminergic tone in healthy women during a sexual conditioning paradigm was manipulated. First, results demonstrated that DA receptor antagonism reduced sexual stimulation-induced genital sexual arousal, emphasizing the importance of DA in unconditional responding to sexual stimulation. However, contrary to the expectations, no differences in conditioned genital responding were seen between the Placebo and Haloperidol

condition after the acquisition phase. Both conditions demonstrated only slight conditioned genital response, but only after correcting for age, prior experience with vibrostimulation and for the difference in genital response towards the CSs during the preconditioning phase. Regarding ratings of affective value, contrary to the expectations no differential responding towards CS+ and CS- after the acquisition phase could be detected, and contrary to the hypothesis, no differences therein were seen between conditions. For ratings of subjective sexual arousal, women in both the Placebo and Haloperidol condition demonstrated increased ratings towards the CS+ on the first trials of the extinction phase. However, the conditions did not differ in this conditioned response.

Results from the present study suggest that DA availability indeed contributes to unconditioned behavioural responses to sexual rewarding stimuli. This is in accordance with previous work that showed that DA systems are involved in (sexual) reward signalling (Both et al., 2005; Brom et al., 2014a; Georgiadis & Kringelbach, 2012; Oei et al., 2012). Quite intriguing is the finding that DA down-regulation did not seem to affect subsequent conditioned genital response and conditioned subjective sexual arousal. However, it is important to keep in mind only very weak conditioned genital responding was seen, making it not straightforward to conclude that administration of the DA receptor antagonist haloperidol did not influence conditioned sexual response in healthy women. This is also evidenced by the data on the magnitude of the genital conditioned response. This finding of only mild conditioned genital response is surprising, especially when considering similar parameters to those of previous research were used, when evience for genital conditioning effects were found (Both et al., 2008, 2011; Brom et al. 2014b,c). Compared to this previous research, in the present study women in both conditions rated the US as less pleasant and less sexually arousing. Although we do not have a clear explanation for why women experienced the

US as less sexually arousing and why no robust conditioned genital response could be detected in the Placebo condition, we should mention that sexually conditioned genital responses have generally been found to be small (Brom et al., 2014a; Hoffmann, Janssen & Turner, 2004; O'Donohue & Plaud, 1994). Of importance, women in the Placebo condition had significantly more experience with genital vibrostimulation. Since the attribution of incentive salience is the product of previous experience (i.e. learned associations; habituation) interacting with someone's genetic propensity and neurobiological state (Flagel et al., 2011), it could be that the US was less effective and rewarding for participants in the Placebo condition. The above makes clear that further replications of the sexual conditioning results in independent samples is highly important.

Although administration of haloperidol resulted in an attenuated unconditioned genital response, this did not seem to affect the perceived pleasantness or sexual arousability of the US, or the magnitude of conditioned subjective sexual arousal. Moreover, in an earlier study on sexual response (Both et al., 2005), levodopa seemed only to increase T reflex magnitude in response to sexual stimulation in men (and not in women), whereas genital and subjective sexual arousal were not affected by levodopa. This suggests that subjectively reported feelings may not be affected by phasic DA signalling. The conscious awareness of a motivational state may be dissociable from the underlying motivational processes (Berridge, 1996; Berridge & Kringelbach, 2008). Moreover, the fact that sexual response systems can diverge has long been recognized, especially in women (Chivers et al., 2010; Laan & Everaerd, 1995; Laan et al., 1995). Women can show increases in VPA while no increases in self-reported sexual arousal are observed, or vice versa.

Several limitations of the current study should be noted. Although we may assume that the administration of 3mg haloperidol 3h before the start of the experimental procedure indeed effectively inhibited dopaminergic tone

(Nordstrom et al, 1992; Darby et al., 1995; Liem-Moolenaar et al., 2010; Oei et al., 2012), also reflected by the difference in reported adverse effects between the haloperidol and placebo conditions, future studies should incorporate additional sensitive measurements of drug induced CNS effects (Liem-Molenaar et al., 2010), to assure testing during maximal plasma concentrations. Moreover, since haloperidol exhibits polypharmacology (i.e. it may affect multiple receptor proteins in the nervous system; Seeman, 2002; Videbaeck et al., 2001), future studies on sexual reward learning in humans, should preferably make use of positron emission tomography and selective ligands in order to be able to attribute its effects to action on D2 receptors. Second, the present study sample exclusively comprised women. Results from a fMRI study by Klucken et al. (2009) on sexual conditioning revealed stronger conditioned activation in the amygdala, thalamus and occipital cortex in men compared to women. The researchers considered these results to be in line with other findings (Gutiérrez & Domjan, 1997; Pfaus et al., 2001), and subsequently suggested that men are more receptive to conditioning of sexual arousal than women. In addition, research has demonstrated that gender differences in the number of DA neurons are influenced by several factors, including sex chromosome complement (Lombardo et al., 2012), the presence of the SRY gene (Dewing et al., 2006) and gonadal hormones. Moreover, it is suggested that testosterone regulates incentive sensitivity through interactions with mesolimbic DA pathways (Hermans et al., 2010; Wood, 2008). Additionally, previous studies have reported conflicting results about the effects of DA on female sexual motivation in animals and humans (Both et al., 2005). This and present findings make clear that future research on the role of DA in sexual learning in both sexes in humans is warranted, as these findings may help in the understanding of the biological mechanisms underpinning addictive behaviours and how these may affect vulnerability to drug abuse or the development of sexual dysfunctions in men and women. In the present study almost all participants

used hormonal contraception. It is known that hormonal contraception may have an influence on the neurochemical regulation of dopaminergic midbrain areas involved in neurobiological processes, herewith affecting reward learning (Brom et al., 2014a; Pletzer & Kerschbaum, 2014; Sotomayor-Zarate et al., 2014). Therefore, future studies on sexual reward learning in women, should preferably include a larger sample of women, in order to investigate the influence of hormonal contraceptives on sexual reward learning. Second, in the present study, vaginal photoplethysmography was used as indicator of physiological sexual arousal. Vaginal engorgement, however, is only one of many co-occurring processes during the sexual arousal response. Functional imaging studies on the role of DA in sexual reward learning in healthy men and women may provide complementary insight in neurochemical mechanisms involved in sexual behaviours, which may help foster potentially critical insights in the aetiology of disorders in sexual motivation. Since sexual arousal can eventually result in overt behaviour such as approach and consumption (Dekker & Everaerd, 1989), future studies should also incorporate a behavioural task to assess automatic action tendencies (Brom et al., 2014b; Wiers et al., 2010). Lastly, another limitation of the present study is the absence of a between-subjects (unpaired) control group. Such a control group would help to distinguish learning about the CS+ and the CS- (Domjan, 2010; Hoffmann et al. 2014). Therefore, making use of such a control group in future research is desirable.

In conclusion, the current study is the first that investigated the role of DA in human sexual reward learning. The present results do not indicate an effect of DA antagonism on conditioned sexual response in women. However, effects of inhibiting dopaminergic tone with a DA antagonist (Haloperidol) were seen in the magnitude of unconditional genital responding to sexual stimulation. Future studies on the role of DA in human sexual reward learning

are warranted, while facilitation as well as impairment of sexual reward learning is relevant in the context of treatment of hypo- and hypersexual desire disorder.

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Chapter 4

Evidence for Persistence of Sexual Evaluative Learning Effects

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Abstract

Research demonstrated that genital arousal and enhanced positive affect towards neutral stimuli due to sexual conditioning did not extinguish during a brief extinction phase. Possible resistance to extinction of conditioned human sexual response has not been studied using extensive extinction trials. Healthy sexually functional men (N= 34) and women (N=32) participated in a differential conditioning experiment, with neutral pictures as conditioned stimuli (CSs) and genital vibrostimulation as unconditioned stimulus (US). Only one CS (the CS+) was followed by the US during the acquisition phase. Men and women rated the CS+ as more positive compared to the CS- during all 24 extinction trials, and demonstrated a slight tendency to approach the CS+ directly after the extinction procedure. Participants rated the CS+ as more sexually arousing than the CS- during 20 extinction trials. No evidence was found for conditioned genital sexual response. The results provide evidence that conditioned sexual likes are relatively persistent, also at the behavioural level.

4.1. Introduction

In the aetiology of sexual dysfunction, such as paraphilia, hypersexuality and related sexual disorders, basic learning processes like classical conditioning are hypothesized to play a pivotal role. In classical conditioning, a neutral stimulus (NS) is repeatedly paired with an unconditioned stimulus (US) (Pavlov, 1927), and eventually the NS is able to trigger the same reaction as the US (Pavlov, 1927; Bindra, 1974). The NS is now called the conditioned stimulus (CS) and the reaction to the CS is called the conditioned response (CR). Research has demonstrated conditioned sexual arousal responses in animals (Pfaus et al., 2012), and recently, some notable studies have demonstrated conditioned sexual arousal responses in humans (for a review see Brom et al., 2014a). Generally, when the CS is repeatedly presented without the US, and the CS no longer predicts the aversive or appetitive outcome (Delamater, 2004), this will result in a loss of conditioned responding (i.e. *extinction*). *Extinction* learning has obvious clinical relevance, since it is thought to be the core mechanism for therapeutic interventions such as exposure therapy (Hermans et al., 2006; Rescorla, 2001; Myers, Carlezon & Davis, 2011). In therapeutic protocols, unwanted emotional responses to specific cues are lessened or inhibited by repeated or prolonged exposure to the cue in absence of the rewarding or aversive event it used to predict. In general, this results in a decrease in the magnitude or frequency of the emotional response.

As a result of classical conditioning, a CS can also acquire the hedonic valence of the US. This form of learning involves the transfer of affective value to an initially neutral stimulus as a result of its contingent presentation with (dis)liked stimuli, and is called evaluative conditioning (De Houwer, Thomas & Baeyens, 2001; Hermans et al., 2002). While in classical conditioning the CS elicits a US expectancy and CR (i.e. signal learning), in evaluative learning it is thought that the CS automatically evokes the representation of the US (Díaz, Ruiz & Baeyens, 2005). Research suggests that although extinction procedures

do eliminate the expressions of US expectancy, extinction procedures do not change the expressed valence of a CS, and as a result, exposure treatment is often unsuccessful in reducing acquired subjective (dis-) likes (Baeyens, et al., 1992; de Houwer, et al., 2001). Experimental studies on conditioned sexual response demonstrated that conditioned genital responses and subjective affect do not extinguish (Both et al., 2011; Brom et al., 2014b), suggesting resistance to extinction of appetitive conditioned sexual response. This is highly clinically relevant, because when conditioned valence and possibly genital arousal are relatively resistant to extinction procedures, then a combination of extinction with some other intervention (e.g. counter conditioning) would presumably be more effective than extinction alone in the treatment of paraphilia, hypersexuality and related sexual disorders. In addition, from fear research and research on disgust it is known that affective evaluations of the CS that persist after extinction of US expectancies are associated with the return (renewal) of conditioned responses (Dirikx, et al., 2007; Hermans et al., 2005; Viar-Paxton & Olatunji, 2012). However, despite the fact that it will likely yield important knowledge about mechanisms underlying sexual motivation and related disorders such as hypo- and hypersexuality, there is only limited empirical research on conditioning of sexual arousal, and research on sexual extinction learning in humans is even scarcer. Only few studies have juxtaposed sexual conditioning in men and women in appetitive paradigms, with mixed results (Hoffmann, Janssen & Turner, 2004; Klucken et al., 2009; Brom et al., 2014b), and none of them investigated extinction of conditioned sexual responses systematically in men and women, making use of the same paradigm, using extensive extinction trials.

Evaluative conditioning paradigms differ from traditional classical conditioning paradigms and it is argued that the parametric differences explain why evaluative learning appears to be resistant to extinction (see Vansteenwegen et al., 2006). Genuine sensitivity to extinction can be observed

making use of classical conditioning procedures that demonstrate conditioning of autonomic responses, as it is thought that the observed resistance to extinction in evaluative conditioning paradigms is produced by demand artifacts or consistency effects (Vansteenwegen et al., 2006). Therefore, to investigate resistance to extinction of different measures of conditioned sexual response, in the present study a differential (autonomic) conditioning paradigm was applied in sexual functional men and women. Genital vibrotactile stimulation served as US, and two neutral pictures served as CS. It was expected that after repeated pairing of the CS and US, genital blood flow would be higher following the picture that was paired with the vibrotactile stimulation (CS+), compared to following the picture that was not paired with the US (CS-). In addition, it was expected that the CS+ would elicit more positive affective value and higher subjective sexual arousal as compared to the CS-. Resistance to extinction was studied by inclusion of a large series of extinction trials (Vansteenwegen et al., 2006). Based on evaluative conditioning theory (de Houwer, et al., 2001) it was expected that genital responses and sexual arousal ratings would show a loss of conditioned responding, while valence ratings (conditioned positive affect) would show no loss. Since affect ratings may be susceptible to demand characteristics, in addition a task was included to assess implicit approach and avoidance tendencies towards the CSs (Wiers et al., 2010; Cousijn, Goudriaan & Wiers, 2011). It was predicted that repeated associations between the CS+ and the vibrotactile stimulation would result in a stronger approach tendency to this CS+, compared to other stimuli, even after a large number of extinction trials.

4.2. Method

4.2.1. Participants

In total 34 men and 32 women (all sexually active) participated in the research, of which 26 participants were students. Participants were paid (€35,-) or received course credit for their participation. Participants between the age of 18 and 45 were recruited through (online) advertisements. Because of the used stimuli, only participants with a heterosexual orientation were included. Exclusion criteria were: sexual problems, a Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV) diagnosis of an affective or psychotic disorder or abusive drug use, pregnancy or breastfeeding, a medical illness or use of medication that could interfere with sexual response, and sexual fetishes or abnormal sexual preferences. In addition, participants reporting a history of sexual abuse and related subsequent psychological problems were also excluded. Before participation all subjects received written information, including a description of the procedure, the vibrotactile stimulation, and the genital response measurement. Women were not tested during menstruation. Confidentiality, anonymity, and the opportunity to withdraw from the experiment without penalty were assured to all participants. The study was approved by the Ethical Committee of the Medical Center.

4.2.2. Design and conditioning procedure

The experimental design involved differential conditioning with one stimulus (the CS+) being followed by genital vibrostimulation (US) during the acquisition phase, whereas the other stimulus (CS-) was never followed by genital vibrostimulation. Which of the two stimuli served as the CS+ was counterbalanced across participants. During the whole experiment measurements of genital arousal were recorded. During the preconditioning-,

and extinction- phases ratings of subjective affect and subjective sexual arousal were collected. For a schematic overview of the procedure see Figure 1. In the preconditioning phase, participants saw four nonreinforced presentations of the CS+ and four presentations of the CS- for 9s each. Subsequently, in the acquisition phase the contingency between CS+ and US was learned: the CS+ and CS- were presented 10 times each and the CS+ was always followed by the US. The extinction phase consisted of 24 unreinforced CS+ presentations and 24 CS- presentations. There were two random orders for each phase; with the restriction of only two successive presentations of each CS. Half of the participants saw the pictures in order 1, the other half in order 2. There was no interval between the preconditioning, acquisition, and extinction phases. During the whole procedure inter-trial intervals (ITIs) were 20, 25, or 30s. The order of the length of the ITI was random, with the restriction of only two successive lengths. The basic design for testing conditioning effects was a 2 (CS+ vs. CS-) x 24 (trial) within subjects design.

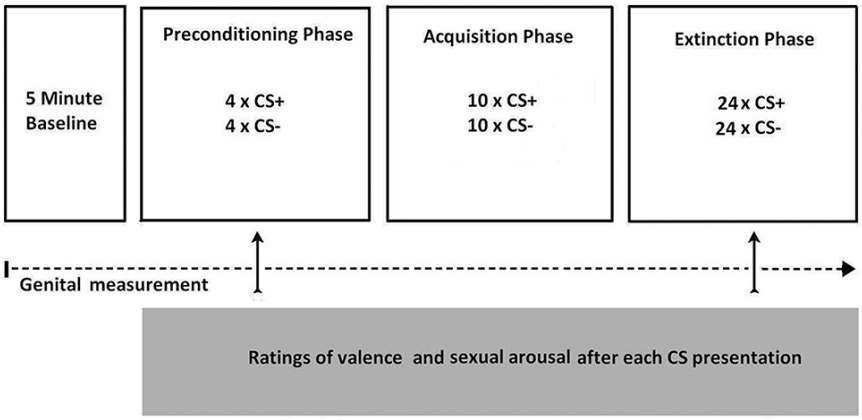

Figure 1. Schematic representation of the conditioning procedure and extinction phase.

4.2.3. Materials, Apparatus, and Recording

Stimulus Materials. Two neutral pictures of pictorial faces as used by Both et al. (2011) and Brom et al. (2014b) served as CS+ and CS-. The pictures differed with regard to details of the drawings, like the nature of the hat, and the glasses the figure was wearing. Male participants were presented with cartoon-like drawings of a female character; women were presented with cartoon-like drawings of a male character. The background and size of the pictures were equal. The CSs were shown in the middle of a computer monitor, approximately 1.5 m in front of the participant. The size of the presented pictures was 14 X 21 cm. During intervals between the pictures, a white screen was presented.

Genital vibrostimulation (US). Genital stimulation was provided only during the acquisition phase, 8s following the start of each CS+ for 2s. For male participants, the vibrotactile genital stimulation was administered by means of a ring-shaped vibrator just below the coronal ridge. For women, a small hands-off vibrator (2 cm diameter) was used (Laan & van Lunsen, 2002). The vibrator was placed on the clitoris using a lycra panty that had an opening for the vaginal plethysmograph. The participants were instructed to place the vibrator in such a way it was *most sexually stimulating*.

Male genital sexual arousal. An indium/gallium-in-rubber penile gauge assessed changes in penile circumference (Bancroft, Jones, & Pullen, 1966; Janssen, Prause, & Geer, 2007). Participants were clearly instructed to place the gauge midway along the penile shaft, making use of an instruction model. Participants were then asked to go over the instructions that were given just before, to assure that they would place the devices correctly. Changes in electrical output caused by expansion of the gauge were recorded by a

continuous DC signal. The Indium-Gallium penile gauges were disinfected after each use, according to Sekusept plus disinfection procedure (MedCaT B.V.).

Women's genital arousal. Vaginal photoplethysmography assessed vaginal pulse amplitude (VPA) (Laan, Everaerd & Evers, 1995). The photoplethysmograph is a menstrual tampon-sized device containing an orange-red light source and a photocell (Manufactured by the technical support department, department of Psychology, University of Amsterdam). The light source illuminates the capillary bed of the vaginal wall and the phototransistor responds to the light backscattered by the vaginal wall and the blood circulating within it. When the signal is connected to an alternating current (AC) amplifier, vaginal pulse amplitude is measured, which reflects the phasic changes in vaginal engorgement accompanying each heartbeat, with larger amplitudes reflecting higher levels of vaginal vasocongestion. VPA is a sensitive, specific, and reliable measure of increases in vaginal vasocongestion in response to sexual stimulation (Laan & Everaerd, 1998). The VPA signal was sampled at 100Hz with a Keithley KPCI3107 A/D converter, running on a Windows2000 PC system. Depth of the probe and orientation of the light emitting diode were controlled by a device (a 6- X 2-cm plate) attached to the cable within 5 cm of the light sensor. The photoplethysmograph was disinfected at the medical centre by means of a plasma sterilization procedure between uses. Plasma sterilization is a highly effective method for the complete removal of all organic (and certain in-organic) material. Genital response was measured continuously during resting baseline, preconditioning, acquisition and extinction phases.

Subjective Ratings. Ratings of affective value, sexual arousal and US expectancy were collected during the preconditioning- and extinction phase. Participants were first asked to rate, after each CS presentation, the affective value of the CSs by answering the question "*What kind of feeling does this picture*

evoke in you?" on a seven-point Likert scale on a keyboard that varied from *very negative* to *very positive*. Then, subjective sexual arousal was rated by answering the question "*How sexually arousing is this picture to you*?" on a seven-point scale that varied from *not sexually arousing at all* to *very sexually arousing*. The questions were presented at the monitor 1 second following the end of picture presentation. The time the question was shown was paced by the participant's response; the time to respond was maximally 11 seconds. When the participant answered the first question, the next question was presented after 15 seconds.

Approach avoidance task (AAT, see Cousijn et al., 2011; E-prime 2.0 Software, Psychology Software Tools, Inc)**.** This task assesses approach and avoidance motivational processes by requiring participants to respond to irrelevant feature of pictures by either pulling a joystick handle toward them or by pushing it away. The amount of time required to execute these actions is the dependent variable. After the extinction phase, participants were presented with the CS+ and CS- pictures from the experiment, as well as neutral pictorial objects and cartoon faces resembling the CSs. The CS+ and CS- were presented 80 times each, 40 times in push- and 40 times in pull-format. Likewise, other test trials consisted of 80 presentations of CS alike pictorial faces and 80 presentations of pictorial objects. The resulting 320 test trials were presented in semi-random order (at most three similar rotations and image categories in a row) and preceded by 15 practice trials with grey rectangles. The latency was recorded between picture onset and lever response. Literature supports the AAT's validity in measuring approach/avoidance motivational processes (Wiers et al., 2011).

The international index of erectile function (IIEF). This is a validated 15-question questionnaire that examines 4 main domains of male sexual function: erectile function (6 questions, range 0-5), orgasmic function (2 questions, range

0-5), sexual desire (2 questions, range 0-5), and intercourse satisfaction (3 questions, range 0-5). Higher scores indicate better sexual function. Psychometric properties of the IIEF are good (Rosen et al., 1997).

The female sexual function index (FSFI). Women's sexual functioning was assessed by the FSFI (Rosen et al., 2000; Ter Kuile, Brauer & Laan, 2006), consisting of six subscales: desire (two items; range 1–5), arousal (four items; range 0–5), lubrication (four items; range 0–5), orgasm (three items; range 0–5), satisfaction (three items; range 0–5), and pain (three items; range 0–5). A higher score indicates better sexual functioning. The FSFI has good internal reliability and is able to differentiate between clinical samples and nondysfunctional controls.

Exit interview. Participants were asked, among others things, about their reactions to the experimental procedure, the use of the genital device, and their evaluation of the genital vibrostimulation. For instance, participants were asked to what extent they liked the vibrostimulation. This could be rated at a 5-point scale ranging from (1) not pleasant at all, to (5) very pleasant. Likewise, participants were asked how sexually aroused they became by the vibration.

4.2.4. Procedure

After participants had given informed consent, they were tested individually by a trained experimenter of the same sex, in a sound-attenuated room. Participants were instructed that the purpose of the experiment was to measure physiological responses to different pictures and to sexual vibrotactile stimuli. They were told that during picture viewing, brief periods of vibrotactile stimulation would be provided. After instructions were given, the experimenter left the room to allow the participant to insert the vaginal probe, or place the penile gauge privately. Further instructions were given through an intercom and

through written instructions on the monitor. Then a 5-minute resting period followed, during which a neutral film was played and baseline measurements of genital response were collected during the last 2 minutes. After the baseline period, the preconditioning, acquisition, and extinction phases followed. Immediately after the experimental procedure had finished and after the participant removed the genital devices and was fully dressed again, the AAT was presented in the experimental room. Lastly, after completion of this task, participants completed privately a questionnaire about demographics, sexual orientation and sexual functioning (e.g., the International Index of Erectile Function (IIEF; Rosen et al., 1997; Rosen, 1998); and the Female Sexual Function Index (FSFI; Rosen et al., 2000; Ter Kuile, Brauer & Laan, 2006). Finally, an exit interview questionnaire was administered. Participants were asked about their reactions to the experimental procedure, the use of the genital device, and their evaluation of the vibrotactile stimulus.

4.2.5. Data Reduction, Scoring and Analysis

A software program (VSRRP98) developed by the Technical Support Department of Psychology (University of Amsterdam) was used to reduce the genital data. The software program enables off-line graphical inspection of the data. Artifacts in the channel monitoring penile circumference and VPA can be caused by movements of the lower part of the body, and for VPA by voluntary or involuntary contractions of the pelvic muscles. These artifacts can be readily detected by the eye in that they show an extreme change in the signal. Artifacts in the penile circumference signal were deleted by hand, and for the VPA signal specialized build-in software was used for artifact deletion. After artifact removal, mean penile circumference or mean VPA level during the 2-minute resting baseline period was calculated. Based on previous studies (Both et al., 2011: Brom et al., 2014b) genital responses to the CSs were scored in three latency windows: during 4-8, 9-12 and 13-16 seconds following CS onset,

respectively FIR (first interval response), SIR (second interval response) and TIR (third interval response). The timeframe of SIR and TIR were included to analyze genital responding during and following (expected) US delivery. For FIR, SIR and TIR, change scores were calculated for each CS presentation by subtracting mean genital resting baseline from genital measurements following CS presentation. Preconditioning and acquisition phases were both analyzed as a whole, whereas the extinction phase was analyzed in steps of 5, 10, 20 and 24 trials at a time, in order to determine thoroughly when extinction of conditioned responding occurred.

Direct gender comparison of genital responses cannot be made because of the use of different measures to assess genital response. Therefore genital data for men and women was analyzed separately, and effects were tested with repeated measures univariate analysis of variance procedures (General Linear Model in SPSS), with Stimulus and Trial as within-subject factors. Analyses of subjective measures were conducted for men and women combined, with Gender as between subjects factor. The Greenhouse–Geisser correction was applied to adjust for violation of the sphericity assumption in testing repeated measures effects. Preconditioning, acquisition, and extinction phases were analyzed separately. Effect sizes are reported as proportion of partial variance (η_p^2) (Cohen, 1988).

Data from the AAT were corrected for outliers (see Cousijn, Goudriaan & Wiers, 2011). The bias score was calculated by subtracting median approach RT from median avoid RT for each image category. The subtraction resulted in a bias score for CS+ images, CS- images, CSs alike images and neutral images for each participant. A positive bias score indicated a relatively faster approach compared to avoid RTs, whereas a negative score indicated a relatively faster avoid compared to approach RTs for the concerned image category. A positive bias score will be referred to further as an approach-bias

and a negative bias score as an avoid-bias. AAT bias scores were analyzed using standard analysis of variance (ANOVA).

With a chosen *p*-value of 0.05, a power of 80% and an effect size of 0.5, a minimal number of 26 subjects was needed for within-subject effects (Cohen, 1988). Since we only explored possible gender differences, it was sufficient to include a minimum of 30 women and 30 men for these within subjects analyses.

Variable	Men (N= 34)		Women (N= 32)		
	M	**SD**	**M**	**SD**	***p***
Age (years)	23.66	4.44	26.13	7.17	*.09*
Sexual Functioning (IIEF/ FSFI-score)	36.66	6.66	27.28	3.35	
Prior experience vibrostimulation	1.71	0.91	3.38	1.26	*<.01**
Pleasantness US	2.91	1.22	3.00	0.80	*.73*
US perceived as sexually arousing	2.71	1.14	2.59	0.76	*.64*
Declared Sexual Arousal	2.06	0.92	2.16	0.88	*.66*
Strongest genital reaction	22.24	26.14	27.88	23.25	*.36*
Erotic fantasies	2.32	1.04	2.03	1.06	*.26*

Table 1. Descriptive subject variables for men and women. Notes: IIEF= *International Index of Erectile Function* (Rosen et al., 1997; Rosen, 1998); FSFI= *Female Sexual Function Index* (Rosen et al., 2000; Ter Kuile, Brauer & Laan, 2006). Questions from the Exit interview, Scales: Prior experience vibrostimulation: 1 (never) – 5 (very often); Pleasantness US: 1 (not pleasant at all) - 5 (very pleasant); US perceived as sexually arousing: 1 (not sexually arousing at all) – 5 (very sexually arousing); Declared Sexual Arousal (in response to US): 1 (no sexual arousal at all) – 5 (much sexual arousal); Strongest genital reaction in %; Erotic fantasies during the experiment: 1 (not at all) – 5 (very much); * $p < .05$.

4.3. Results

The results for men's and women's genital data are based on 31 men and 30 women. Due to error data from three male participants and one female participant was lost. In addition, an experimental error caused an invalid baseline value for one woman, resulting in outliers on all subsequent measurements in all phases. The results for the subjective ratings are based on 34 men and 32 women. With respect to the Approach and Avoidance Task, due to technical error, data of one female participant were lost. For study sample characteristics, see Table 1.

4.3.1. Genital Sexual Arousal

Preconditioning phase. Analyses were conducted to verify equal levels of penile circumference and VPA in response to the CS+ and CS- during the preconditioning phase. For all latency windows (FIR, SIR and TIR), no difference in penile circumference following presentation of the CS+ and CS- was found, all *p*s > .29. For FIR and TIR no difference in VPA following the CS+ or CS- was found, all *p*s > .32, but for TIR a significant main effect for Stimulus was found, $F(1, 28) = 4.98$, $p < .04$, $\eta_p^2 = .15$, indicating differential responding towards the CS+ and CS- in the preconditioning phase. As can be seen in Figure 3, the CS+ elicited higher VPA as compared to the CS-.

Acquisition phase. Penile circumference and VPA in response to the vibrotactile stimulation during the acquisition phase was determined in order to verify whether the sexual stimulus elicited genital responses. Genital responses in the second and third latency windows (SIR, TIR) were considered as responses to the vibrotactile stimulation.

Men. Figure 2 summarizes penile circumference (SIR) to CS+ and CS- across trials. The analysis of penile circumference in the acquisition phase revealed a main effect of Stimulus, FIR F (1, 30)= 10.74, p< .01, η_p^2 = .26, SIR F(1, 30)= 85.37, p< .01, η_p^2 = .74; TIR F(1, 30)= 8.23, p< .01, η_p^2 = .22, meaning the vibrostimulation resulted in a genital response, as can be seen in Figure 2. In line with former studies (Brom et al. 2014b; Brom et al. *under review*), penile circumference to CS- was larger as compared to CS+. No effects for Trial were observed, all ps> .10, and no significant 2 (Stimulus) × 10 (Trial) interaction was found, all ps> .38.

Women. In line with previous studies (Both et al, 2011), the 2 (Stimulus) X 10 (Trial) repeated measures ANOVA of VPA FIR during the acquisition phase revealed no significant main effect for Stimulus, p= .21. In line with the hypothesis, on SIR and TIR this analysis did yield a significant main effect for Stimulus, SIR, F(1, 28)= 4.27, p< .05, η_p^2 = .13, TIR, F(1, 29)= 21.87, p< .01, η_p^2 = .43, indicating genital responding. As can be seen in Figure 3, the vibrostimulation resulted in a genital arousal response. On TIR also an interaction for Stimulus X Trial was seen, F(4, 124)= 3.17, p< .02, η_p^2 = .10, indicating differentiation between genital responding to CS+ plus vibrostimulation and CS- over trials.

Extinction phase.

Men. As can be seen in Figure 2, men showed no increase of differential responding towards CS+ and CS- after the acquisition phase. Analysis of penile circumference during the preconditioning phase (Mean precon trial 1-4) and the first extinction trial, yielded no interaction effect for Stimulus X Trial, all ps> .19. Analysis of the first extinction trial, yielded no significant main effect for Stimulus, all ps> .30, indicating no conditioned responding. Analysis of the first

five extinction trials yielded no significant main effect for Stimulus on all time latencies, all *p*s> .24. In addition, no main effect for Trial was detected, all *p*s>.54, and subsequently, no interaction effect for Stimulus X Trial, all *p*s> .64. This indicates that there was no difference in penile responding towards the CS+ and CS-, and the pattern of responding did not change across extinction trials. Subsequent analyses of 10, 20 and 24 extinction trials neither yielded significance for Stimulus, Trial or Stimulus X Trial, all *p*s> .21

Women. As can be seen in Figure 3, women showed no increase of differential responding towards CS+ and CS- after the acquisition phase. Analysis of VPA during the preconditioning phase (Mean precon trial 1-4) and the first extinction trial, yielded no interaction effect for Stimulus X Trial, FIR *p*= .99, SIR *p*= .68, TIR *p*= .24. Analysis of the first extinction trial revealed no significance for Stimulus on FIR, *p*= .75, and SIR, *p*= .78, but revealed a trend for Stimulus on TIR, $F(1, 29)= 3.36$, $p< .08$, indicating slight differential responding towards the CS+ and CS- on this first extinction trial. However, analysis of the first five extinction trials yielded no significant main effect for Stimulus on all time latencies, all *p*s> .33, indicating no conditioned responding. In addition, no main effect for Trial was detected, all *p*s> .69. Not surprisingly, subsequent analyses of 10, 20 and 24 extinction trials neither yielded significance for Stimulus, Trial or Stimulus X Trial, all *p*s> .14.

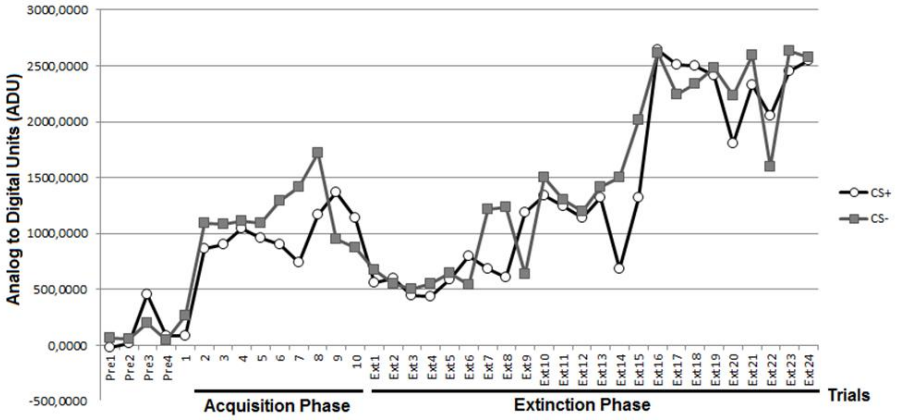

Figure 2. Mean penile circumference change scores during the third interval response window (TIR) following CS+ and CS- during the preconditioning phase, acquisition phase and extinction phase. Note that during the acquisition phase, the response represents responding to the CS+ plus the US. [2]

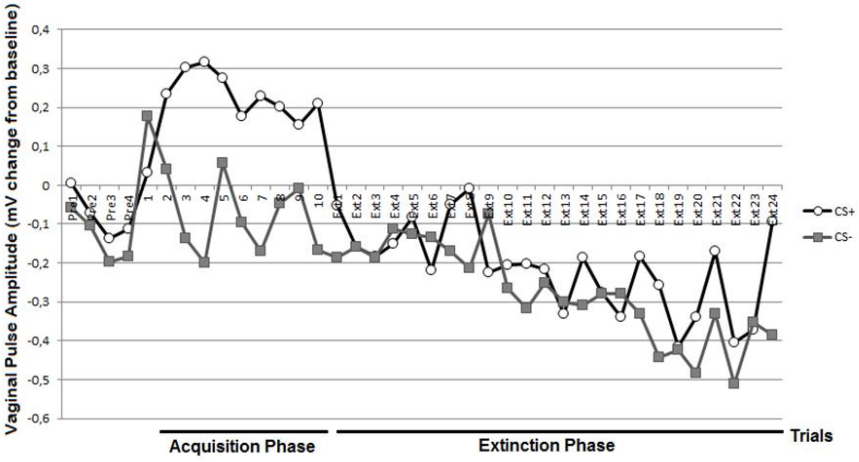

Figure 3. Mean vaginal pulse amplitude (VPA) change scores during the third interval response window (TIR) following CS+ and CS- during the preconditioning phase, acquisition phase and extinction phase. Note that during the acquisition phase, the response represents responding to the CS+ plus the US.

[2] Since not all indium-gallium gauges could be calibrated before data collection, results were calculated in digital output units.

4.3.2. Subjective Measures

Preconditioning phase. The 2 (Stimulus) X 4 (Trial) X 2 (Gender) repeated measures ANOVA was conducted to verify equal levels of subjective responses to the CS+ and CS- during the preconditioning phase. For affective value and subjective sexual arousal, no difference in responding following presentation of the CS+ and CS- was found between men and women, all *p*s > .17.

Extinction Phase.

Subjective Affect. As can be seen in Figure 4, men and women showed an increase of differential responding towards CS+ and CS- after the acquisition phase. Analysis of the affective value ratings during the preconditioning phase (Mean precon trial 1-4) and the first extinction trial, revealed a significant interaction effect for Stimulus X Trial, $F(1, 59) = 28.76$, $p < .01$, $\eta_p^2 = .33$. No differences were seen between men and women, reflected by the non-significant Stimulus X Trial X Gender interaction, $p = .36$. In line with the hypothesis, the analyses of the first five extinction trials yielded a significant main effect for Stimulus, $F(1, 58) = 26.72$, $p < .01$, $\eta_p^2 = .32$, indicating conditioning effect. Men and women showed stronger positive affect towards the CS+ after the acquisition phase. This 2 (Stimulus) X 5 (Trial) X 2 (Gender) repeated measures ANOVA yielded also a significant interaction effect for Stimulus X Trial, $F(3, 187) = 2.80$, $p < .04$, $\eta_p^2 = .05$, indicating extinction effect. No differences in differential responding were seen between men and women, as reflected by the non-significant interaction effects for Stimulus X Gender, $p = .41$, and Stimulus X Trial X Gender, $p = .58$. Analysis of the first 10 extinction trials yielded a main

effect for Stimulus, $F(1, 58) = 20.22$, $p < .01$, $\eta_p^2 = .26$, and for Stimulus X Trial, $F(6, 372) = 3.20$, $p < .01$, $\eta_p^2 = .05$. Again, no differences between men and women were seen, Stimulus X Gender, $p = .77$; Stimulus X Trial X Gender, $p = .21$. This indicates that up to 10 extinction trials men and women showed more positive affect towards the CS+. However, as reflected by the significant Stimulus X Trial interaction, this difference in rated subjective affect between CS+ and CS- gradually decreased across extinction trials. Subsequent analysis of the first 20 extinction trials also yielded a main effect for Stimulus, $F(1, 53) = 9.75$, $p < .01$, $\eta_p^2 = .16$, and for Stimulus X Trial, $F(11, 575) = 3.17$, $p < .01$, $\eta_p^2 = .06$. The interaction for Stimulus X Trial X Gender, approached significance, $F(11, 575) = 1.75$, $p = .06$. Analysis of all 24 extinction trials still revealed a main effect for Stimulus, $F(1, 37) = 6.36$, $p < .02$, $\eta_p^2 = .15$, indicating conditioned responding during 24 extinction trials, and for Stimulus X Trial, $F(11, 391) = 1.96$, $p < .04$, $\eta_p^2 = .05$, indicating a reduction of differential responding towards the CS+ and CS-. Again, a trend was seen for the interaction Stimulus X Trial X Gender, $F(11, 391) = 1.62$, $p = .09$. Additional analysis of the first extinction trial, revealed a main effect for Stimulus, $F(1, 61) = 27.77$, $p < .01$, $\eta_p^2 = .31$, with no differences between men and women, $p = .13$, whereas analysis of the last extinction trial did only yield a trend for Stimulus, $p < .07$, with again no differences between men and women, $p = .35$.

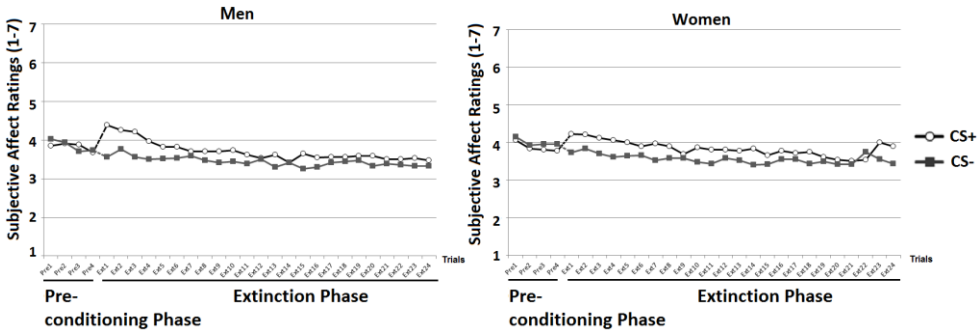

Figure 4. Subjective affect ratings following the CS+ and CS- during the preconditioning phase and extinction phase for men (left) and women (right).

Subjective Sexual Arousal. Figure 5 shows the ratings of subjective sexual arousal across all trials. In line with the expectations, analysis of the first extinction trial, revealed a main effect for Stimulus, $F(1, 64) = 11.32$, $p < .01$, $\eta_p^2 = .15$, with no differences in this conditioned responding between men and women, $p = .49$. Analysis of the ratings of subjective sexual arousal during the preconditioning phase (Mean precon trial 1-4) and the first extinction trial, revealed a main effect for Stimulus, $F(1, 64) = 14.84$, $p < .01$, $\eta_p^2 = .19$, and an interaction effect for Stimulus X Trial, $F(1, 64) = 15.49$, $p < .01$, $\eta_p^2 = .20$. No differences were seen between men and women, reflected by the non-significant Stimulus X Gender and Stimulus X Trial X Gender interactions, both ps $> .50$. In line with the hypothesis, the analyses of the first five extinction trials yielded a significant main effect for Stimulus, $F(1, 61) = 8.32$, $p < .01$, $\eta_p^2 = .12$, indicating conditioning effect. Men and women showed stronger subjective sexual arousal towards the CS+ after the acquisition phase. This 2 (Stimulus) X 5 (Trial) X 2 (Gender) repeated measures ANOVA did not yield a significant interaction effect for Stimulus X Trial, only a trend was seen, $F(3, 175) = 2.48$, $p < .07$, indicating no extinction of conditioned responding. No differences in differential responding were seen between men and women, as reflected by the

non-significant interaction effects for Stimulus X Gender, p= .38, and Stimulus X Trial X Gender, p= .57. Analysis of the first 10 extinction trials also yielded a main effect for Stimulus, $F(1, 60)= 7.83$, $p< .01$, $\eta_p^2 = .12$. No significant interaction effect was found for Stimulus X Trial, p=. 11, indicating no extinction of conditioned differential responding towards the CS+ and CS-. Again, no differences between men and women were seen, Stimulus X Gender, p= .62; Stimulus X Trial X Gender, p= .18. This indicates that up to 10 extinction trials men and women declared to find the CS+ more sexually arousing as compared to the CS-. Analysis of the first 20 extinction trials also yielded a main effect for Stimulus, $F(1, 57)= 4.22$, $p< .05$, $\eta_p^2 = .07$. Now also a significant interaction effect for Stimulus X Trial was seen, $F(6, 143)= 2.39$, p= .02, $\eta_p^2 = .04$, indicating extinction of conditioned responding. Again, no differences between men and women were seen, all ps > .18. Finally, analysis of all 24 extinction trials did not yield a main effect for Stimulus anymore, p= .15, nor for Stimulus X Trial, p= .16, indicating that over all 24 extinction trials no conditioned responding could be detected. No differences were seen between men and women, all ps > .65. Analysis of the last extinction trial did also not yield significance for Stimulus, p= .11, indicating no differential responding on this last extinction trail towards the CS+ and the CS-, with no differences therein between men and women, p= .29.

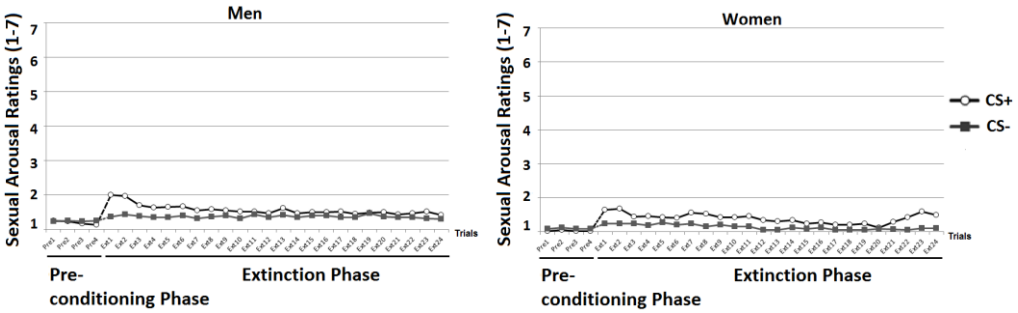

Figure 5. Ratings of subjective sexual arousal following the CS+ and CS- during the preconditioning phase and extinction phase for men (left) and women (right).

4.3.3. Approach Avoidance Tendencies

Differences in AAT bias scores were analysed with mixed ANOVA with Gender as between-subject factor and Image as within-subject factor (CS+, CS-, CS alike and neutral objects). A trend was found for Image, $F(3, 168)= 2.39$, $p< .08$, $\eta_p^2 = .04$, suggesting that participants differed in approach and avoidance tendencies towards the different stimuli. No differences therein were seen between men and women, as reflected by the non-significant Image X Gender interaction, $p=.65$.

In additional analysis, CS+ bias scores were compared with the bias scores of CS-, CS alike and Neutral images. Since multiple comparisons are done, tests were conducted using Bonferroni adjusted alpha levels of $p<.017$ (.05/3). The analysis yielded no difference between CS+ bias scores and CS- bias scores, $p= .32$, with no differences therein between men and women, $p= .78$. Likewise comparison between CS+ and CS-alike bias scores neither yielded significance for Stimulus, $p= .51$, with no difference between men and women, $p= .63$. However, the analysis of CS+ and Neutral images yielded a strong trend for Stimulus, $F(1, 63)= 5.26$, $p= .02$, $\eta_p^2 = .08$, indicating that participants were

slightly faster in approaching CS+ images as compared to neutral images, see Figure 6.

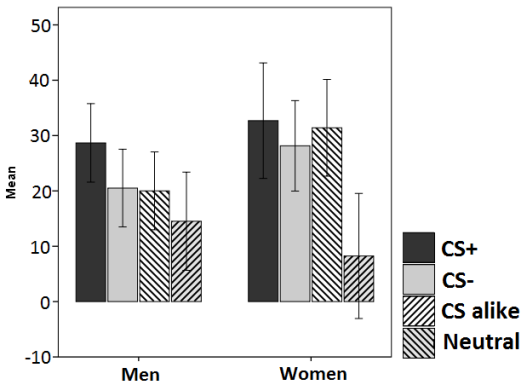

Figure 6. Mean Approach Avoidance Task (AAT) bias score for CS+, CS-, CS alike and neutral images in men and women (ms with standard error bars). A positive score indicates faster reaction times on pull (approach) trials compared to push (avoid) trials.

4.4. Discussion

The present study provides evidence that sexual evaluative learning effects are difficult to modify through the procedure of extinction, at least in an appetitive sexual paradigm, in healthy sexually functional men and women. The results revealed that extinction trials eventually reduced subjective sexual arousal towards the CS+. Importantly, appetitively conditioned subjective affect and approach tendencies towards the CSs, seem to be even more persistent. These findings are consistent with prior research suggesting that acquired likes and dislikes are resistant to extinction (Vansteenwegen et al., 2006; Gawronski, Gast & de Houwer, 2014). The results from the AAT demonstrated that the pairing of the CS+ with the sexual vibrotactile stimulus did still result in slight

approach tendencies towards this CS+ in men and women, even after a very extensive extinction phase. Apparently the CS+ retained sexual affective value to elicit approach. It is highly reasonable that the AAT measured learned evaluative sexual likes that survived extinction, as participants 'knew' that the US would not be presented during this task since all genital devices were removed before completion of this task. Therefore, the observed slight approach tendencies towards the CS+ must have been due to its hedonic value rather than its predictive value.

The absence of a conditioning effect for the genital measure does not hamper any conclusions about the persistence of sexual evaluative learning effects. The absent conditioned genital arousal response that was observed in men is in line with former research from our lab (Brom et al., 2014b). It seems that the combination of neutral CSs and a vibrotactile US is insufficient to elicit conditioned genital sexual responding in men. However, surprisingly, women also did not show robust genital conditioned response in the present study. This is remarkable, given that similar parameters to those of previous research were used (Both et al., 2008, Both et al., 2011; Brom et al. 2014b). Although there is no clear explanation for this, it should be mentioned that sexually conditioned responses have generally been found to be small (O'Donohue & Plaud, 1994; Hoffmann, Janssen & Turner, 2004). Nevertheless, future studies may provide further evidence for the hypothesis that sexual evaluative conditioning is indeed distinct from other forms of sexual conditioning by using sexually relevant pictures as CSs instead of neutral pictures, so as to increase the chances of observing genital conditioning effects in men and women. In a previous study (Brom et al., 2014b), making use of the same paradigm but with sexually relevant CSs as the only difference, robust conditioned genital and subjective sexual arousal and subjective value was observed in men. Future studies should investigate how persistent conditioned

genital responses are and to what extent and in which period of time they eventually will (or will not) extinguish.

Although this study highlights the potential shortcoming of extinction in reducing learned sexual likes, there are some limitations of this study that must be considered before definitive inferences can be made. First, it is possible that the observed absence of conditioning effect and subsequent extinction effect of the genital measure is due to measurement error rather than a genuine lack of conditioning. For future studies it would be interesting to include a between subjects (unpaired) control group. With such a control group one can determine even more precisely whether and what learning has occurred. For instance, the possibility of sensitization of sexual arousal would translate into increased genital responses across trails, and not in differential responding towards the CS+ and CS- per se (Domjan, 2010; Hoffmann, et al., 2014). Second, the AAT was administered at the end of the experimental conditioning procedure. It is therefore unclear whether the results would have been different when the task was administered before or after acquisition, or after extinction. Third, no subjective measure of US expectancy was included in the present study. Earlier research (Brom et al., 2014b) revealed that different response systems do not always behave in synchrony with each other in a sexual conditioning procedure: US expectancy, subjective sexual arousal and subjective affect may go hand in hand during this process of conditioning in men, whereas in women subjective sexual arousal does not seem to increase affective value, or vice versa. And lastly, and clinically relevant, the present study investigated only newly acquired sexual evaluative learning and relatively short-term effects within one experimental session.

Despite these limitations, former research on conditioned sexual response has not incorporated such extensive extinction manipulations. Findings from the present study and from earlier research (Brom et al., 2014b) suggest that although an extinction procedure may reduce the CS-US

contingency, learned sexual evaluations may be difficult to modify through this procedure. Therefore, in the treatment of sexual disorders with a learned component, like hypersexuality or paraphilia, unwanted but persistent subjective sexual evaluations may be better targeted by interventions such as counterconditioning or the deployment of emotion regulation strategies. In counterconditioning, the CS is paired with a stimulus evoking a response that is incompatible with the original unconditioned response, thereby altering the valence of a stimulus (Baeyens, et al., 1992). Although the effects of counterconditioning on evaluative learning has received little attention in the literature, research on appetitive conditioning in the domain of food stimuli has shown that counterconditioning is more effective than extinction alone in changing evaluations of the CS (Van Gught et al., 2010). In addition, research on the deployment of an emotion regulation strategy (i.e. attentional deployment) during sexual conditioning, demonstrated that emotion down-regulation affected extinction of conditioned evaluative sexual learning effects in men, and in women down-regulation resulted in attenuated conditioned approach tendencies towards the CSs (Brom et al., 2015b).

Quite intriguing is the finding that making use of exact the same procedure, but with a painful stimulus as US and erotic pictures as CSs, in a parallel aversive sexual conditioning paradigm, sexual evaluative learning effects were not difficult to modify through the procedure of extinction (Brom et al., 2015a*)*. In that study, next to attenuated female genital and subjective sexual arousal towards the CS+ on the first few extinction trials, men and women showed less positive affect towards the CS+ up to 10 extinction trials. However, for all measures extinction of conditioned responding was seen within 10 extinction trials, and no conditioned behavioural avoidance tendencies were seen towards the CS+ after the extinction phase. This suggests that appetitive and aversive sexual extinction learning seem to encompass distinct processes and are not organized in the same fashion. Research has

demonstrated appetitive - aversive interactions in dopamine neurons in the brain reward system: when a neuron is excited by an aversive CS it is inhibited by an appetitive CS or vice versa (Matsumoto & Hikosaka, 2009; Bouton & Peck, 1992; Nasser & McNally, 2012). In addition, recruitment of the relevant motivational system (appetitive vs aversive) is dependent on the US. Painful stimulation (e.g. electric shock) can selectively activate the aversive system, whereas sexual stimulation (e.g. genital vibrostimulation) will activate the appetitive system. However, since erotic pictures were used as CSs in the parallel aversive study (Brom et al., 2015a), these pictures most likely automatically recruited the appetitive motivational system. In addition, the painful stimulation that served as US most likely recruited the aversive motivational system. Since the two motivational systems oppose each other, a CS which excites one motivational system will inhibit the other. In other words, a conditioned excitor of one motivational system is functionally equivalent to a conditioned inhibitor of the other, and prior appetitive sexual learning could have interfered or augmented sexual aversive learning (Nasser & McNally, 2012). In the present study neutral pictures were used as CSs, and as a consequence, only the appetitive motivational system was recruited by the US, and no prior learning interfered with CR acquisition. The question remains if the mechanisms described here would be effective in clinical practice in the treatment of sexual motivation disorders such as female sexual interest/arousal disorder or sexual aversion. Likewise, it will be of interest to investigate counterconditioning in sexual motivation disorders at the other end of the spectrum, such as hypersexuality or paraphilia. Early studies on the 'treatment' of homosexuality or undesired sexual behaviours have applied counterconditioning procedures in order to shape sexual behaviour (see Brom et al., 2014a for a review). Although these uncontrolled (case) studies yielded mixed results, it would be of interest to systematically investigate the effect of counterconditioning on appetitively learned sexual evaluative effects, in healthy

participants but eventually also in clinical samples. Like applied in fear research and treatment (Wolpe, 1968), counter conditioning in the treatment of paraphilia for instance, would consist of encouraging patients to visualize or imagine the targeted sexually-arousing stimulus while pairing this stimulus with an aversive stimulus (e.g. an aversive smell, a loud noise or a disgusting (mental) image) until eventually the most sexually arousing image no longer yields sexual response, also at the evaluative level. These possible mechanisms in changing unwanted sexual CRs remain important directions for future research, including the neural mechanisms for appetitive-aversive interactions that are poorly understood, as it will likely yield important knowledge which may help in the development of clinical treatments for maladaptive sexual behaviours, including paraphilias and deviant sexual preferences that manifest perturbed motivation, but also for the more prevalent sexual desire and arousal disorders.

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Chapter 5

Extinction of Aversive Classically Conditioned Human Sexual Response

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Abstract

Research has shown that acquired subjective likes and dislikes are quite resistant to extinction. Moreover, studies on female sexual response demonstrated that diminished genital arousal and positive affect towards erotic stimuli due to aversive classical conditioning did not extinguish during an extinction phase. Possible resistance to extinction of aversive conditioned sexual responses may have important clinical implications. However, resistance to extinction of aversive conditioned human sexual response has not been studied using extensive extinction trials. The aim of the present study is to investigate resistance to extinction of aversive conditioned sexual responses in sexually functional men and women. Penile circumference and vaginal pulse amplitude were assessed and ratings of affective value and subjective sexual arousal were obtained. Also a stimulus response compatibility task was included to assess automatic approach and avoidance tendencies. A differential conditioning experiment was conducted, with two erotic pictures as CSs and a painful stimulus as USs. Only one CS (the CS+) was followed by the US during the acquisition phase. Conditioned responses were assessed during the extinction phase. Men and women rated the CS+ more negative as compared to the CS-. During the first trials of the extinction phase vaginal pulse amplitude was lower in response to the CS+ than in response to the CS-, and on the first extinction trial women rated the CS+ as less sexually arousing. Intriguingly, men did not demonstrate attenuated genital and subjective sexual response. Aversive conditioning, by means of painful stimuli, only affects sexual responses in women, whereas it does not in men. Although conditioned sexual likes and dislikes are relatively persistent, conditioned affect eventually does extinguish.

5.1. Introduction

Lack of sexual interest is the most common sexual problem among women and is often accompanied by the experience of low sexual arousal (Mercer et al., 2003; West et al., 2008). Although low sexual interest is most common in women, it may also manifest itself in men as psychogenic erectile dysfunction (Georgiadis & Kringelbach, 2012). Disorders in sexual motivation, like hypoactive sexual desire disorder (HSDD), are linked to complex interplay of psychological and biological factors, and are generally considered as difficult to treat. Regrettably, empirically validated treatments are lacking (ter Kuile, Both & van Lankveld, 2010). Insight in the underlying mechanisms of sexual motivation is essential to understand these disorders, and may guide treatment thereof.

In the aetiology of sexual dysfunction, basic learning processes like conditioning are hypothesized to play a pivotal role. Learning about sexual cues may encompass learning of positive expectations of pleasure and sexual reward, but may also include the learning of negative expectations (Ågmo, 1999). According to incentive motivation models, sexual motivation is the result of the interplay of a sensitive internal sexual system with external motivational stimuli or the mental representation thereof. External stimuli that can promote motivation are called incentive stimuli (Ågmo, 1999; Singer & Toates, 1987). Sexual incentives are stimuli that elicit sexual responses and approach behaviour. Hence, sexual motivation may be investigated by studying responses within various response systems involved in sexual behaviour (Both et al., 2005a; Robbins & Everitt, 1999). Sexual arousal can be seen as an evolutionary preserved emotion (Everaerd, 1988), which is characterized by specific bodily reactions, like enhanced genital blood flow, by preparation of behavioural action (Both et al., 2005b), and by the experience of feelings of lust, excitement, and sexual desire. In addition, sexual arousal can eventually result in overt behaviour such as approach and consumption (Dekker & Everaerd, 1989).

Important to note is that in women, genital arousal is not per se accompanied by subjective desire or arousal (Laan & Everaerd, 1995a, b; Laan, Everaerd & Evers, 1995). However, the agreement between physiological sexual arousal and subjective report seems to increase as a function of the strength of the physiological response (Laan, Everaerd & Evers, 1995). The incentive motivation model of sexual desire suggests that the experience of sexual desire may follow rather than precede sexual excitement, and suggests that sexual desire emerges following sexual arousal initiated by a sexually meaningful stimulus (Laan & Both, 2008).

The motivational valence of incentive stimuli can be unconditioned (primary) or conditioned (secondary) as a result of associative leaning (Di Chiara, 1995). In associative learning processes like classical conditioning, a neutral stimulus (NS) is repeatedly paired with an unconditioned stimulus (US) (Pavlov, 1927), and eventually the NS is able to trigger the same reaction as the US (Pavlov, 1927; Bindra, 1974). The NS is now called the conditioned stimulus (CS) and the reaction to the CS is called the conditioned response (CR). It is suggested that as a result of aversive conditioned learning, sexual arousal may decrease after negative sexual experiences, such as sexual assault or repeated experiences with painful coitus (van Berlo & Ensink, 2000; Brauer et al., 2007). Therefore, the role of aversive experiences and memories in sexual desire and arousal problems is likely to be important. However, there is only limited empirical research on classical conditioning of sexual arousal in humans, while it is likely to yield important knowledge about mechanisms underlying sexual motivation and related disorders such as hyposexuality.

Despite its clinical relevance, studies on aversive sexual conditioning in humans are scarce in the literature (Brom et al., 2014a; O'Donohue & Plaud, 1994; Quinn, Harbison & McAllister, 1970; Rosen & Kopel, 1997). In a study in women (Both et al., 2008a), making use of an erotic picture as the CS and a painful stimulus at the wrist as the US, diminished genital arousal and increased

negative affect in response to the CS+ were observed. This was the first study that provided evidence for attenuation of sexual response in women by aversive conditioning. Generally, when the CS is repeatedly presented without the US, and the CS no longer predicts the aversive or appetitive outcome (Delamater et al., 2004), this will yield in a loss of conditioned responding. Intriguing, in this study (Both et al., 2008a) conditioned genital responses and subjective affect did not diminish significantly during the extinction phase suggesting resistance to extinction. This *extinction* learning process has obvious clinical relevance, since it is thought to be the core mechanism for therapeutic interventions such as exposure therapy (Hermans et al., 2006; Myers, Carlezon & Davis, 2001; Rescorla, 2001). In such therapeutic protocols, conditioned responses are lessened or inhibited by repeated or prolonged exposure to a cue (the CS) in absence of the event it used to predict (the US). This results in a decrease in the magnitude or frequency of the conditioned response (CR). This observation that conditioned subjective affect did not extinguish is in line with research on evaluative conditioning, which has shown that acquired subjective likes and dislikes are relatively resistant to extinction (DeHouwer, Thomas & Baeyens, 2001). Research has demonstrated that exposure treatment is often unsuccessful in reducing feelings of dislike (Baeyens et al., 1992). In classical conditioning the CS elicits a US expectancy and CR (i.e. signal learning), whereas in evaluative learning, it is thought that the CS automatically evokes the representation of the US (Diaz, Ruiz & Baeyens, 2005). As a result, evaluative learning effects are difficult to modify through the procedure of extinction alone. Therefore, research on resistance to extinction of different measures of conditioned sexual responses is needed as it may have important clinical implications.

At present, it is unclear if gender differences in sexual conditionability and in resistance to extinction of aversive conditioned responses do exist. Only few studies have addressed sexual conditioning in both men and women in

appetitive paradigms (Brom et al., 2014b; Hoffmann, Janssen & Turner, 2004; Klucken et al., 2009), with mixed results, and none have examined aversive conditioning in both sexes. In addition, results from animal studies are also mixed. Some have demonstrated that intense electrical shock inhibits male rat sexual performance (Beach et al., 1956), while other research demonstrated that the pairing of a CS with a painful shock can also induce copulation in noncopulating male rats (Barfield & Sachs, 1968; Caggiula & Eibergen, 1969; Crowley, Popolow & Ward, 1973). Recently, an intriguing study by Farmer et al. (2014) demonstrated that inflammatory pain reduces sexual motivation in female but not in male laboratory mice.

To investigate resistance to extinction of aversively conditioned sexual responses systematically, in the present study an aversive conditioning paradigm was applied in sexually functional men and women. A painful electric stimulus at the wrist served as US, and two erotic pictures served as CS. It was expected that after repeated pairing of the CS and US, genital blood flow would be attenuated following the erotic picture that was paired with the painful electric stimulus (CS+), compared to following the erotic picture that was not paired with the US (CS-), at least in women. In addition, it was expected that pairing of the erotic CS+ with painful stimulation would result in more negative affective value and lower sexual arousability ratings of the CS+ as compared with the CS-. Resistance to extinction was studied by inclusion of a large series of extinction trials (Vansteenwegen et al., 2006). Based on evaluative conditioning theory it was expected that genital responses and sexual arousal ratings would show a loss of conditioned responding, while valence ratings (conditioned positive and negative affect) would show no loss. Since affect ratings are susceptible to demand characteristics, in addition a stimulus response compatibility task was included to assess implicit approach and avoidance tendencies towards the CSs. In this task, participants responded to stimuli either by pushing or pulling a lever. Former research has shown that

participants are faster to respond to negatively valenced stimuli when pushing the lever away (avoid) than when pulling it toward them (approach) but were faster to respond to positive stimuli by pulling than pushing the lever (Chen & Bargh, 1999). It was predicted that repeated associations between the CS+ and the painful stimulation will result in a stronger avoid tendency to this CS+, compared to other stimuli on this task, even after the large number of extinction trials.

5.2. Method

5.2.1. Participants

Research participants were 38 men and 34 women. Participants were paid €35,- or received course credit for their participation. Participants between the age of 18 and 45 were recruited through online advertisements at social networks and through advertisements at the Leiden University Medical Centre, and at the Universities of Leiden and Amsterdam. Due to the nature of the CSs only participants with a heterosexual orientation were included. Exclusion criteria were: sexual problems, a Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV) diagnosis of an affective or psychotic disorder or abusive drug use, pregnancy or breastfeeding, a medical illness or use of medication that could interfere with sexual response, and sexual fetishes or abnormal sexual preferences. In addition, participants reporting a history of sexual abuse and related subsequent psychological problems were also excluded. Before participation all subjects received written information, including a description of the procedure, the pain stimulus, and the genital response measurement. Women were not tested during menstruation. Confidentiality, anonymity, and the opportunity to withdraw from the experiment without penalty were assured to all participants. The study was approved by the Ethical Committee of the Medical Center.

5.2.2. Design

The experimental design involved differential conditioning with one stimulus (the CS+) being followed by the painful stimulation (US) during the acquisition phase, whereas the other stimulus (CS-) was never followed by a painful shock. Which of the two pictures served as the reinforced CS (the CS+), or the nonreinforced CS (the CS-) was counterbalanced across participants. During the whole experiment measurements of genital arousal were recorded. During the preconditioning-, and extinction- phases ratings of subjective affect and subjective sexual arousal were collected. In the preconditioning phase, participants saw four nonreinforced presentations of the CS+ and four presentations of the CS-, for 9s each (see Figure 1). Subsequently, in the acquisition phase the contingency between CS+ and US was learned: the CS+ and CS- were presented 10 times each and the CS+ was always followed by the US. The US was delivered for 50ms, starting 8s after the onset of the CS+. The extinction phase consisted of 24 unreinforced CS+ presentations and 24 unreinforced CS- presentations. There were two random orders for each phase; with the restriction of only two successive presentations of each CS. Half of the participants saw the pictures in order 1, the other half in order 2. There was no interval between the preconditioning, acquisition, and extinction phases. During the whole procedure inter-trial intervals (ITIs) were 20, 25, or 30s. These intervals allowed sufficient time for the genital response not to interfere with subsequent stimulus presentation, since data from our laboratory has shown that vaginal blood engorgement is a relatively slow physiological response (Both et al., 2008a,b). The order of the length of the ITI was random, with the restriction of only two successive lengths. Procedure, timing of US, and ITI were adapted from previous studies that demonstrated conditioned sexual response (Both et al., 2008a, 2011). The basic design for testing conditioning effects was a 2 (CS+ vs. CS-) x 24 (trial) within subjects design.

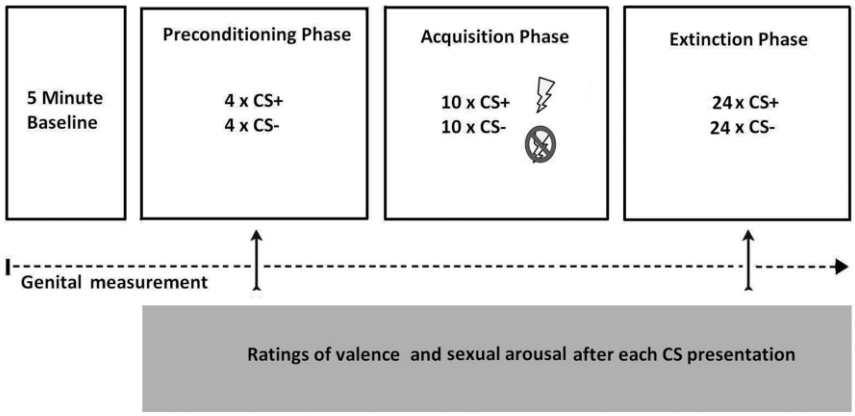

Figure 1. Schematic representation of the conditioning procedure and extinction phase.

5.2.3. Materials

Stimulus Materials

Conditioned Stimuli Two explicit erotic pictures as used by Both et al. (2008a) served as CSs. Both pictures portrayed a nude heterosexual couple engaging in sexual intercourse with the woman in superior position. The pictures differed with regard to the male and female actors involved. The CSs were shown in the middle of a computer monitor, approximately 1.5 m in front of the participant. The size of the presented pictures was 14 X 21 cm. During the intervals between the pictures, a white screen was presented. One of the pictures (CS+) was followed by the US, while the other picture (CS−) was never followed by the US. Assignment of the pictures as CS+ and CS− was counterbalanced across participants.

Pain stimulus The pain stimulus (US) was an electrocutaneous stimulus with a duration of 50 milliseconds, delivered by a safe muscle stimulation apparatus (Digitimer, DS7A, Digitimer Ltd, Hertfordshire, England) with an isolation unit. The pain stimulus was administered by electrodes, fastened at the

wrist of the nondominant arm. The pain stimulus produces a painful, stinging sensation, and has been used in several experimental studies (Both et al., 2008a; Brauer et al., 2007; Effting & Kindt, 2007). The intensity of the US was set at a level that the participant described as *painful and demanding some effort to tolerate*.

Physiological Measures

Male genital sexual arousal Changes in circumference of the penis were measured using an indium/gallium-in-rubber penile gauge (Bancroft, Jones & Pullan, 1966). Increases in penile circumference result in a corresponding change in resistance. Changes in electrical output caused by expansion of the gauge were recorded by a continuous DC signal. Participants were instructed to place the gauge midway along the penile shaft. The Indium-Gallium penile gauges were disinfected after each use, according to Sekusept plus disinfection procedure.

Women's genital sexual arousal was measured using a vaginal photoplethysmograph assessing vaginal pulse amplitude (VPA) (Laan, Everaerd & Evers, 1995). The photoplethysmograph is a menstrual tampon-sized device containing an orange-red light source and a photocell. The light source illuminates the capillary bed of the vaginal wall and the blood circulation within it. VPA can be measured when the signal is connected to an alternating current amplifier. VPA reflects the phasic changes in vaginal engorgement accompanying each heartbeat, with larger amplitudes reflecting higher levels of vasocongestion. Depth of the probe and orientation of the light emitting diode were controlled by a device (a 6- X 2-cm plate) attached to the cable within 5 cm of the light sensor. Participants were instructed to insert the photoplethysmograph until the plate touched their labia. The vaginal photoplethysmograph was sterilized by plasma sterilization. Genital response was measured continuously during resting baseline, preconditioning-, acquisition- and extinction phases.

Subjective Measures

Subjective ratings of affective value and sexual arousal in response to the CSs were collected during the preconditioning- and extinction phase. Participants were asked to rate, after each stimulus presentation, the affective value of the CSs by answering the question "*What kind of feeling does this picture evoke in you*?" With a keyboard the question could be answered on a seven-point Likert-scale that varied from *very negative* to *very positive*. Subjective sexual arousal was rated by answering the question "*How sexually arousing is this picture to you*?" The question could be answered on a seven-point scale that varied from *not sexually arousing at all* to *very sexually arousing*. The first question was presented at the monitor one second following the end of picture presentation. The time the question was shown was paced by the participant's response; the time to respond was maximally 11 seconds. When the participant answered the first question, the next question was presented after 15 second. For the benefit of the conditioning procedure, a constant time interval between the CS presentations was kept. After the participants answered the questions, a white screen remained until the next picture was presented.

Other measures

Approach Avoidance Task (AAT) After the extinction phase, participants performed the implicit approach/avoidance task (Cousijn, Goudriaan & Wiers, 2011). This task assesses approach and avoidance motivational processes by requiring participants to respond to irrelevant features of the pictures by either pulling a joystick handle toward them or by pushing it away. Pulling and pushing the joystick gradually increased and decreased image-size. All images were rotated 3° left or right. Image content was irrelevant to the task: participants were instructed to pull or push a joystick in response to rotation direction. Half the participants pushed images rotated left and pulled images rotated right, while the other half received opposite instructions. Pulling and

pushing the joystick gradually increased and decreased image-size. Appetitive images should facilitate pull (i.e., approach) responses, and aversive images should facilitate (push) (avoidance) responses. The amount of time required to execute these actions is the dependent variable. The task was programmed making use of the stimulus presentation program E-prime 2.0. The CS+ and CS- pictures from the experiment were presented, as well as other explicit erotic pictures resembling the CSs (CS-alike), and neutral pictures (i.e., always a man and a women engaged in neutral activity, such as reading or walking). Participants were randomly assigned to one of the two stimulus-response conditions. Literature supports the AAT's validity in measuring approach/avoidance motivational processes (Wiers et al., 2011).

International Index of Erectile Function (IIEF) Male sexual functioning was assessed by the IIEF. This is a validated 15-question questionnaire that examines 4 main domains of male sexual function: erectile function (6 questions, range 0-5), orgasmic function (2 questions, range 0-5), sexual desire (2 questions, range 0-5), and intercourse satisfaction (3 questions, range 0-5) (Rosen et al., 1997; Rosen, 1998). Higher scores (25-30) indicate better sexual function. Psychometric properties of the IIEF are good (Rosen et al., 1997).

The Female Sexual Function Index (FSFI) Female sexual arousal was assessed by the FSFI. (ter Kuile, Brauer & Laan, 2006; Rosen et al., 2000). This is a validated 19-question questionnaire that examines 6 main domains of female sexual function: desire (2 items; range 1–5), arousal (4 items; range 0–5), lubrication (4 items; range 0–5), orgasm (3 items; range 0–5), satisfaction (3 items; range 0–5), and pain (3 items; range 0–5). Higher scores indicate better sexual functioning. Psychometric properties of the FSFI are good (Wiegel, Meston & Rosen, 2005).

5.2.4. Procedure

After participants had given informed consent, they were tested individually by a trained experimenter of the same sex, in a sound-attenuated room. Participants were instructed that the purpose of the experiment was to measure physiological responses to different erotic pictures and to pain stimuli. They were told that during picture viewing, brief periods of painful stimulation would be provided. After extensive instructions were given, the experimenter left the room to allow the participant to insert the vaginal probe, or place the penile gauge privately. The participants were instructed to lay a blanket on their lap after insertion of the probe or placement of the penile gauge, so the experimenter could enter the participant room to attach the pain stimulus electrodes. Subsequently, for each participant individually, the level of the pain stimulus was determined. The participant was exposed to repeated pain stimuli (50ms) of increasing intensity until he/she determined the pain stimulus as *painful and demanding some effort to tolerate*. It was emphasized that the intensity of the stimulus would not be changed during the experiment. Further instructions were given through an intercom and through written instructions on the monitor. Then a 5-minute resting period followed, during which a neutral film was played and baseline measurements of genital response were collected during the last 2 minutes. After the baseline period, the preconditioning, acquisition, and extinction phases followed. Immediately after the experimental procedure had finished and after the participant removed the genital devices and was fully dressed again, the AAT was presented in the experimental room. Lastly, after completion of this task, participants completed privately a questionnaire about demographics, sexual orientation and sexual functioning (e.g., FSFI/IIEF). Finally, an exit interview questionnaire was administered. Participants were asked about their reactions to the experimental procedure, the use of the genital device, and their evaluation of the pain stimulus.

5.2.5. Data Reduction, Scoring, and Analysis

A software program (VSRRP98) developed by the Technical Support Department of Psychology (University of Amsterdam) was used to reduce the genital data. The software program enables off-line graphical inspection of the data. Artifacts in the channel monitoring penile circumference and VPA can be caused by movements of the lower part of the body or by voluntary or involuntary contractions of the pelvic muscles. After artifact removal, mean penile circumference or mean VPA level during the 2-minute resting baseline period was calculated. Based on previous studies (Both et al., 2008a,b; Both, Brauer & Laan, 2011) genital responses to the CSs were scored in three latency windows: during 4-8, 9-12 and 13-16 seconds following CS onset, respectively FIR (first interval response), SIR (second interval response) and TIR (third interval response). This means that in the extinction phase SIR and TIR overlapped with the moment participants were answering the questions. The timeframe of SIR and TIR were included to analyze genital responding during and following (expected) US delivery. For FIR, SIR and TIR, change scores were calculated for each CS presentation by subtracting mean genital resting baseline from genital measurements following CS presentation. Preconditioning and acquisition phases were both analyzed as a whole, whereas the extinction phase was analyzed in steps of 5 trials at a time, in order to determine thoroughly when extinction of conditioned responding occurred.

Direct gender comparison of genital responses cannot be made because of the use of different measures to assess genital response. Therefore genital data for men and women was analyzed separately, and effects were tested with repeated measures univariate analysis of variance procedures (General Linear Model in SPSS), with Stimulus and Trial as within-subject factors. Analyses of subjective measures were conducted for men and women combined, with Gender as between subjects factor. The Greenhouse–Geisser correction was applied to adjust for violation of the sphericity assumption in

testing repeated measures effects. Preconditioning, acquisition, and extinction phases were analyzed separately. Effect sizes are reported as proportion of partial variance (η_p^2) (Cohen et al., 1988).

Data from the AAT were corrected for outliers: Response Times (RTs) below 200ms, above 2000ms and more than 3 standard deviations (SD) above and below the mean were removed for each participant. Error trials were removed. The bias score was calculated by subtracting median approach RT from median avoid RT for each image category. The subtraction resulted in a bias score for CS+ images, CS- images, CSs alike images and neutral images for each participant. Median RTs were used because they are less sensitive to outliers than means (Cousijn, Goudriaan & Wiers, 2011). A positive bias score indicated a relatively faster approach compared to avoid RTs, whereas a negative score indicated a relatively faster avoid compared to approach RTs for the concerned image category. A positive bias score will be referred to further as an approach-bias and a negative bias score as an avoid-bias. AAT bias scores were analyzed using standard analysis of variance (ANOVA).

With a chosen *p*-value of .05, a power of 80% and an effect size of .5, a minimal number of 26 subjects was needed for within-subject effects (Cohen, 1988). Since we only explored possible gender differences, it was sufficient to include a minimum of 30 women and 30 men for these within subjects analyses.

5.3. Results

The results for men's and women's genital data are based on 36 men and 31 women. Data of two men and one woman were lost due to technical error. Data from another female participant were left out, because due to an experimental error this participant did not receive the painful stimulation. This person was removed from all further analysis. Data of another woman were

discarded because genital data were 3 SD above from the Mean. The results for the subjective ratings are based on 35 men and 32 women. For a female participant and for three male participants the subjective data was lost due to technical error. With respect to the Approach and Avoidance Task, due to technical error, data of one female participant were lost.

5.3.1. Study sample characteristics

Men and women did not differ in age, p= .93 (see Table 1 for subject characteristics). For men, the International Index of Erectile Function Questionnaire (IIEF) mean score was 33.53 (SD= 4.57, range 22-41), indicating sexual functioning within the normal range (Rosen, 1998). Mean Female Sexual Function Index (FSFI) score was 26.32 (SD = 3.82, range 15.2–30.6), indicating sexual functioning slightly below the normal range (Rosen et al., 2000). However, 11 women indicated not to have a stable relationship at the time of the study, six participants indicated not having had sexual activity with a partner during the last weeks, and one participant indicated no sexual activity during the last 4 weeks, resulting in a low FSFI score, which may explain the relatively low FSFI group score. Mean FSFI score without participants that indicated no sexual activity with a partner the last 4 weeks was 26.96 (SD 3.19, range 18.30–30.60), indicating sexual functioning within the normal range.

5.3.2. Evaluation and Effect of US

Prior to the experiment, men and women differed in set US intensity, $t(70)$= 3.81, p<. 01, and the evaluation in terms of painfulness thereof, $t(67)$= 9.31, p<. 01. In the exit interview, 84.2% of the male participants and 70.5% of the women rated the US as fairly unpleasant. Of the men, 81.6% and 76.4% of the women rated the US as moderate to very painful. There were no differences between men and women in the way they had experienced the US during conditioning, see Table 1.

Variable	Men (N= 38)		Women (N= 34)		
	M	SD	M	SD	*p*
Age (years)	24.5	5.5	24.7	6.4	*.93*
Sexual Functioning (IIEF/ FSFI-score)	33.5	4.6	26.3	3.8	
Intensity US (mA)	1200.3	244.0	903.9	403.9	*<.001**
Ratings US as painful	6.8	0.4	5.5	0.7	*<.001**
CSs perceived as sexually arousing	3.0	0.9	1.24	0.7	*<.01**
Pleasantness watching CSs	3.2	0.8	2.7	0.8	*.02**
Unpleasantness US	3.3	0.9	3.0	1.0	*.17*
US perceived as painful	3.1	0.7	3.0	0.8	*.31*
US perceived as sexually arousing	1.7	0.9	1.7	0.9	*.98*
Strongest genital reaction	30.5	28.1	39.6	24.8	*.15*
Prior experience erotic material	4.3	0.6	3.6	0.7	*<.001**
Erotic fantasies	2.6	1.1	2.6	1.1	*.89*

Table 1. Descriptive subject variables for men and women. Notes: Scale Rating US as painful (directly after intensity of US was set): 1 (not very painful at all) – 7 (very painful). Questions from the Exit interview: CSs perceived as sexually arousing: 1 (not sexually arousing at all) – 5 (very sexually arousing); Pleasantness watching CSs: 1 (not pleasant at all) - 5 (very pleasant); Scale unpleasantness US: 1 (not unpleasant at all) - 5 (very unpleasant); US perceived as painful (exit interview): 1 (not painful at all) – 5 (very painful); US perceived as sexually arousing : 1 (not sexually arousing at all) – 5 (very sexually arousing); Strongest genital reaction in %; Scale Prior experience erotic material: 1 (never) – 5 (very often); Erotic fantasies during the experiment: 1 (not at all) – 5 (very much); * $p < .05$.

5.3.3. Preconditioning phase

Genital Sexual Arousal Analyses were conducted to verify equal levels of penile circumference and VPA in response to the CS+ and CS- during the preconditioning phase. For all latency windows (FIR, SIR and TIR), no

difference in circumference following presentation of the CS+ and CS- was found, all *p*s > .40, nor in VPA, all *p*s > .11.

Subjective Measures The 2 (Stimulus) X 4 (Trial) X 2 (Gender) Mixed ANOVA was conducted to verify equal levels of subjective responses to the CS+ and CS- preceding the acquisition phase. For affective value and subjective sexual arousal, no difference in responding following presentation of the CS+ and CS- was found between conditions and between men and women, all *p*s > .48. However, for both measures a main effect for Gender was seen, with men rating the CSs as more positive, $F(1, 59)= 9.27$, $p< .01$, $\eta_p^2 = .14$, and as more sexually arousing, $F(1, 64)= 9.09$, $p< .01$, $\eta_p^2 = .12$, as can be seen in Figure 3 and 4.

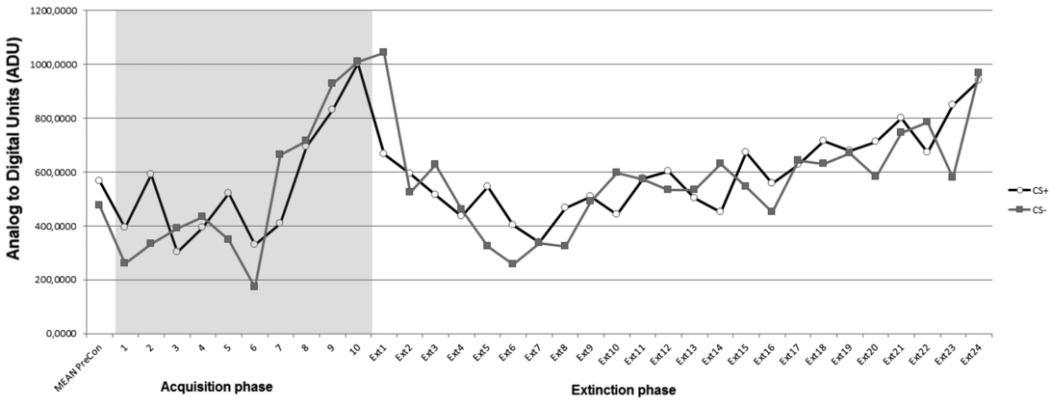

Figure 2. Mean penile circumference change scores during the second interval response window (SIR) following CS+ and CS- during the preconditioning phase, acquisition phase and extinction phase. Note that during the acquisition phase, the response represents responding to the CS+ plus the US.[3]

[3] Since not all indium-gallium gauges could be calibrated before data collection, to avoid bias results were calculated with digital output units.

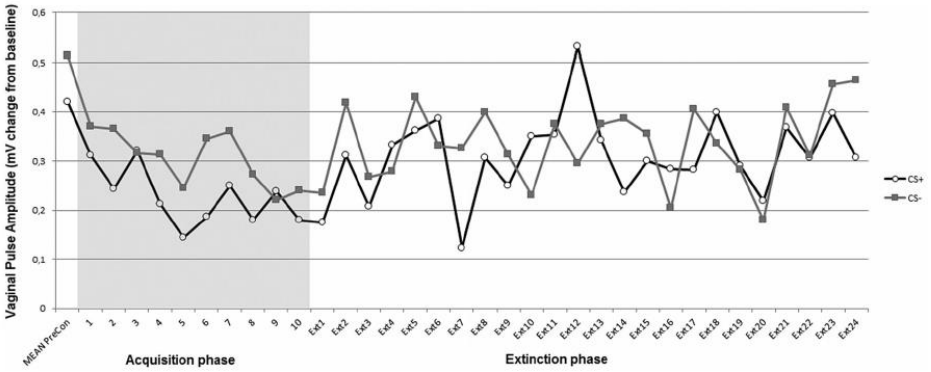

Figure 3. Mean vaginal pulse amplitude (VPA) change scores during the second interval response window (SIR) following CS+ and CS- during the preconditioning phase, acquisition phase and extinction phase. Note that during the acquisition phase, the response represents responding to the CS+ plus the US.

5.3.4. Acquisition phase

Genital Sexual Arousal Penile circumference and VPA in response to the painful stimulation during the acquisition phase was determined in order to verify whether the electric shock diminished genital responses. Genital responses in the second and third latency windows (SIR, TIR) were considered as responses to the painful stimulation.

Men Figure 2 summarizes penile circumference (SIR) to CS+ and CS- across trials. Contrary to expectation, the analysis of penile circumference in the acquisition phase revealed no main effect of Stimulus at all time latencies, FIR, p= .85, η_p^2 <.01; SIR, p= .77, η_p^2 < .01; TIR, p= .76, η_p^2 < .01, meaning the painful stimulus did not result in a decreased genital response. Also, the 2 (Stimulus) X 10 (Trial) interaction was not significant, FIR p= .82, η_p^2 < .01, SIR, p= .81, η_p^2 < .01, and TIR, p= .71, η_p^2 = .01. Analysis of penile circumference during the preconditioning phase (Mean precon trial 1-4) and the first acquisition trial yielded a significant main effect for Stimulus on TIR, F(1,

35)= 4.22, p< .05, η_p^2 = .11. Additional analysis of penile circumference during the preconditioning phase (Mean precon trial 1-4) and the last acquisition trial yielded no main effect for Trial on all time latencies, all ps> .64. In sum, the painful stimulation only resulted in a marginally decrease of penile circumference at the first trials of the acquisition phase as compared with the preconditioning phase. However, as can be seen in Figure 2, after the initial decrease in penile circumference during the first trials of the acquisition phase, contrary to the expectations, a trend was seen for an increase in penile circumference in response to the two CSs on all time latencies.

Women The 2 (Stimulus) X 10 (Trial) repeated measures ANOVA of VPA FIR during the acquisition phase revealed no significant main effect for Stimulus, p= .14, η_p^2 = .08 (see also Both et al., 2008b; Both, Brauer & Laan, 2011). For SIR this analysis yielded a significant main effect for Stimulus, F(1, 27)= 10.92, p< .01, η_p^2 = .29, and for TIR a trend, F(1, 28)= 6.63, p< .07, η_p^2 = .12. As can be seen in Figure 3, the painful stimulation resulted in a robust decreased genital arousal response. On all time latencies, no interaction for Stimulus X Trial was found, FIR, p= .47, η_p^2 = .03; SIR, p= .61, η_p^2 = .03; TIR, p= .80, η_p^2 = .02.

5.3.5. Extinction phase

Genital Sexual Arousal

Men Analysis of the first extinction trial revealed smaller penile responses towards CS+ than to CS-, although this difference only approached conventional level of significance, FIR, F(1, 35)= 3.12, p< .09, η_p^2 = .09; SIR, F(1, 35)= 3.33, p< .08, η_p^2 = .09; TIR, F(1, 35)= 2.85, p= .10, η_p^2 = .08.

Analysis of penile circumference during the preconditioning phase (Mean precon trial 1-4) and the first extinction trial yielded no significant main effect for Stimulus, all *p*s> .45, nor significant interaction effects for Stimulus X Trial, all *p*s >.12. On SIR and TIR this analysis yielded a trend for Trial, SIR $F(1, 35) = 3.36$, $p < .08$, $\eta_p^2 = .09$, TIR, $F(1, 35) = 2.86$, $p < .10$, $\eta_p^2 = .08$. The 2 (Stimulus) X 5 (Trial) repeated measures ANOVA of the first five extinction trials yielded a main effect for Trial approaching significance, on the time latency FIR, $F(2, 78) = 2.85$, $p < .06$, $\eta_p^2 = .08$, and a trend for Stimulus X Trial on SIR, $F(3, 89) = 2.51$, $p = .07$, $\eta_p^2 = .07$, and TIR $F(2, 68) = 2.64$, $p = .08$, $\eta_p^2 = .07$. This indicates there was only a marginally difference in penile responding towards the CS+ and CS-, and this pattern of responding slightly changed across extinction trials, meaning extinction. Analysis of the whole extinction phase yielded no significant interaction effect for Stimulus X Trial, all *p*s> .24. Not surprisingly, additional analysis of the last extinction trial did not yield significance for Stimulus, all *p*s >.52, indicating no conditioned differential responding towards the CS+ and CS-. In sum, the picture that was reinforced by painful stimulation during the acquisition phase did not elicit smaller penile circumference during the extinction phase, although trends in that direction were seen during the first extinction trials.

Women Analysis of VPA during the preconditioning phase (Mean precon trial 1-4) and the first extinction trial yielded a significant main effect for Stimulus on all time latencies, FIR, $F(1, 28) = 11.25$, $p < .01$, $\eta_p^2 = .29$; SIR, $F(1, 28) = 28.04$, $p < .01$, $\eta_p^2 = .50$; TIR, $F(1, 29) = 6.53$, $p < .02$, $\eta_p^2 = .18$. The 2 (Stimulus) X 5 (Trial) repeated measures ANOVA of VPA of the first five extinction trails revealed no main effect of Stimulus on FIR, $F(1, 30) = 0.13$, $p = .73$, $\eta_p^2 < .01$, but yielded a significant main effect of Stimulus on the time

latency SIR, $F(1, 30) = 4.69$, $p < .04$, $\eta_p^2 = .14$, indicating a conditioning effect. Additional analysis of the first 10 extinction trials on SIR also yielded a significant main effect for Stimulus, $F(1, 27) = 4.96$, $p < .04$, $\eta_p^2 = .16$. This means that the picture that was reinforced by painful stimulation during the acquisition phase did elicit lower vaginal pulse amplitudes during the first extinction trials compared to the picture that was not paired with the US, as can be seen in Figure 3. Contrary to the hypothesis however, this analysis did not yield a main effect for Stimulus on the time latency TIR, $p = .44$, $\eta_p^2 = .20$. Although not entirely surprising, since no main effect for Stimulus was found on FIR and TIR, the ANOVA showed no significant interaction effect for Stimulus and Trial on FIR, $p = .15$, $\eta_p^2 = .06$, and TIR, $p = .28$, $\eta_p^2 = .04$. But crucial to the hypothesis this analysis also did not show a significant interaction effect for Stimulus and Trial on SIR, $p = .68$, $\eta_p^2 = .01$, indicating no extinction effect. However, analysis of the whole extinction phase yielded no significant main effect for Stimulus, FIR, $p = .37$, $\eta_p^2 = .04$; SIR, $p = .44$, $\eta_p^2 = .04$; TIR, $p = .47$, $\eta_p^2 = .03$, nor significant interaction effects for Stimulus X Trial on SIR, $p = .48$, $\eta_p^2 = .05$, and TIR, $p = .42$, $\eta_p^2 = .05$, although on FIR a trend was seen, $F(9, 178) = 1.77$, $p < .08$, $\eta_p^2 = .09$. These results indicate that the differential VPA responding towards the picture that was reinforced by painful stimulation during the acquisition phase (CS+) and the picture that was not followed by painful stimulation (CS-) did not endure during the whole extinction phase. The conditioned differential responding gradually decreased across extinction trials, and this is considered as evidence of extinction. Additional analysis of the last extinction trial did not yield significance for Stimulus on FIR, $p = .96$, $\eta_p^2 < .01$, and TIR, $p = .66$, $\eta_p^2 = .01$, indicating extinction of conditioned responding.

However for SIR, a trend for Stimulus was detected, $F(1, 20) = 3.29$, $p < .09$, $\eta_p^2 = .14$, although this finding does not lend itself to unambiguous interpretation as resistance to extinction.

Subjective Measures

Subjective Affect

As can be seen in Figure 4, men and women showed an increase of differential responding towards CS+ and CS- after the acquisition phase. Analysis of the affective value ratings during the preconditioning phase (Mean precon trial 1-4) and the first extinction trial, revealed an interaction effect for Stimulus X Trial, $F(1, 57) = 7.74$, $p < .01$, $\eta_p^2 = .12$. No differences were seen between men and women, reflected by the non-significant Stimulus X Trial X Gender interaction, $p = .35$, $\eta_p^2 = .02$. In line with the hypothesis, the analyses of the first five extinction trials yielded a significant main effect for Stimulus, $F(1, 61) = 6.47$, $p < .02$, $\eta_p^2 = .10$, indicating a conditioning effect. Men and women showed stronger negative affect towards the CS+ after the acquisition phase. The 2 (Stimulus) X 5 (Trial) X 2 (Gender) Mixed ANOVA yielded a significant interaction effect for Stimulus X Trial, $F(3, 196) = 4.91$, $p < .01$, $\eta_p^2 = .07$, indicating extinction effect. No differences in differential responding were seen between men and women, as reflected by the non-significant interaction effects for Stimulus X Gender, $p = .87$, $\eta_p^2 < .01$, and Stimulus X Trial X Gender, $p = .86$, $\eta_p^2 < .01$. Analysis of the first 10 extinction trials yielded a main effect for Stimulus, $F(1, 59) = 4.15$, $p < .05$, $\eta_p^2 = .07$, and for Stimulus X Trial, $F(5, 293) = 4.40$, $p < .01$, $\eta_p^2 = .07$. Again, no differences between men and women were seen, Stimulus X Gender, $p = .69$, $\eta_p^2 < .01$; Stimulus X Trial X Gender, $p = .94$,

η_p^2 < .01. This indicates that up to 10 extinction trials men and women showed less positive affect towards the CS+. However, as reflected by the significant Stimulus X Trial interaction, this difference in rated subjective affect between CS+ and CS- gradually decreased across extinction trials. Analysis of all 24 extinction trials revealed no main effect for Stimulus, p= .32, η_p^2 = .02, but an interaction effect for Stimulus X Trial, F(6, 282)= 3.98, p< .01, η_p^2 = .08, indicating extinction. For Trial a trend was seen, F(3, 152)= 2.14, p= .09, η_p^2 = .04, indicating rated subjective affect changed over trials. Again, no differences were seen between men and women, Stimulus X Gender, p= .90, η_p^2 < .01; Stimulus X Trial X Gender, p= .83, η_p^2 =.01. Additional analysis of the first extinction trial, revealed a main effect for Stimulus, F(1, 63)= 11.55, p< .01, η_p^2 = .16, whereas analysis of the last extinction trial did not yield significance for Stimulus, p= .65, η_p^2 < .01, indicating extinction of conditioned responding.

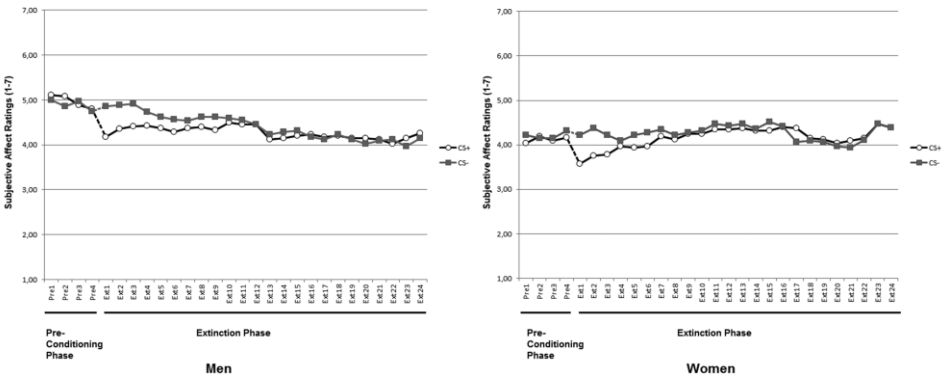

Figure 4. Subjective affect ratings following the CS+ and CS- during the preconditioning phase and extinction phase for men (left) and women (right).

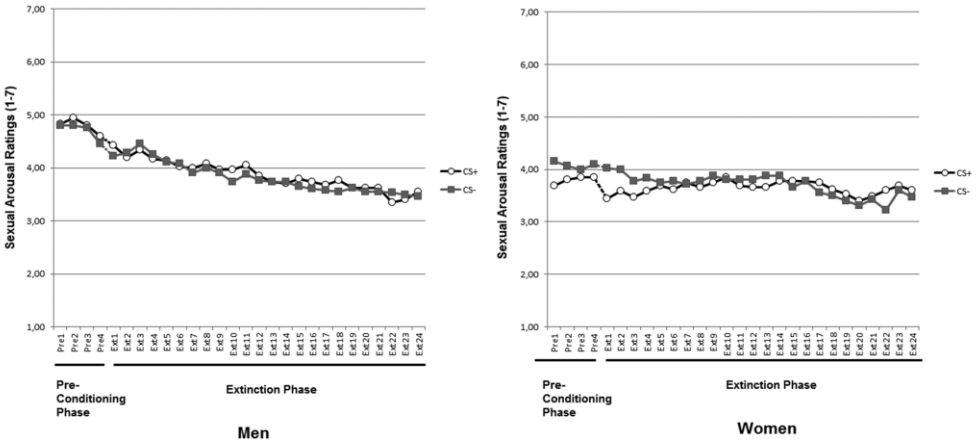

Figure 5. Ratings of subjective sexual arousal following the CS+ and CS- during the preconditioning phase and extinction phase for men (left) and women (right).

Subjective Sexual Arousal

Figure 5 shows the ratings of subjective sexual arousal across all trials. Additional analysis of the first extinction trial, revealed no main effect for Stimulus, p= .26, η_p^2 = .02, although a significant interaction effect for Stimulus X Gender was seen, $F(1, 65)$= 5.29, p= .03, η_p^2 = .08. Men and women differed in conditioned responding on the first extinction trial, with only women showing conditioned differential responding. Inspection of Figure 5 reveals that compared to men, women rated the CS+ as less sexually arousing as compared to the CS-. Analysis of the ratings of subjective sexual arousal during the preconditioning phase (Mean precon trial 1-4) and the first extinction trial, revealed a main effect for Stimulus, $F(1, 64)$= 6.18, p< .02, η_p^2 = .09, but no interaction effect for Stimulus X Trial, p= .49, η_p^2 = .01. No differences were seen between men and women, as reflected by non-significant interactions, Stimulus X Gender, p= .36, η_p^2 = .01, and Stimulus X Trial X Gender, p= .17,

η_p^2 = .03. Contrary to the hypothesis, the analyses of the first five extinction trials did not yield a significant main effect for Stimulus, *p*= .19, η_p^2 = .03. However, the analysis did yield an interaction effect for Stimulus X Trial X Gender approaching significance, *F*(3, 193)= 2.37, *p*= .07, η_p^2 = .04. As can be seen in Figure 5, women demonstrated more differential responding on the first five extinction trials towards CS+ and CS- as compared to men. Analysis of the first 10 extinction trials yielded no significant main effect for Stimulus, *p*= .45, η_p^2 < .01, or significant interaction effects for Stimulus X Trial, *p*= .11, η_p^2 = .03, Stimulus X Trial X Gender, *p*= .27, η_p^2 = .02. Analysis of the last extinction trial did not yield significance for Stimulus, *p*= .40, η_p^2 = .01, indicating extinction of conditioned responding. No differences were seen between men and women on this last extinction trial, as reflected by the non-significant Stimulus X Gender interaction, *p*= .86, η_p^2 < .01.

Approach Avoidance Tendencies

One-sample *t*-tests were used to test if bias scores deviated significantly from zero within each condition, see Table 2. Differences in AAT bias scores were analyzed with a mixed ANOVA with Gender as between-subject factor and Image as within-subject factor with four levels (CS+, CS-, CS-alike and neutral objects). Contrary to the expectations, no main effect was found for Image, *F*(2, 161)= 0.27, *p*= .81, η_p^2 < .01. Participants did not differ in approach and avoidance tendencies across all stimuli. In addition, no interaction effect was found for Image and Gender, *F*(2, 161)= 1.54 *p*= .21, η_p^2 = .02, meaning men and women did not differ in their bias scores.

	Bias Score	M	SD	*p*
Men	CS+	24.5	76.0	*.06*
N= 38	CS-	24.1	67.4	*.03**
	CS-alike	41.6	130.9	*< .05**
	Neutral	37.4	70.5	*.01**
Women	CS+	45.6	51.7	*< .01**
N= 32	CS-	40.9	58.8	*< .01**
	CS-alike	35.5	65.8	*< .01**
	Neutral	22.6	49.9	*< .02**

Table 2. One-sample *t*-test results for Mean Approach Avoidance Task (AAT) bias scores for CS+, CS-, CS-alike and neutral images in men and women. A positive score indicates faster reaction times on approach (pull) trials compared to avoid (push) trials. * $p < .05$.

5.4. Discussion

The present study contributes to the existing and growing literature on learning mechanisms in sexual behaviours, and provides further support for attenuation of sexual response through aversive conditioning, especially in women. The present study replicated the study by Both and colleagues (Both et al., 2008a) who provided the first evidence for the effect of aversive conditioning on female sexual response. However, contrary to the hypotheses, the present study does not provide evidence that sexual evaluative learning effects are difficult to modify through the procedure of extinction alone, at least in an aversive sexual paradigm, in heterosexual healthy men and women. Yet the results do corroborate the notion that conditioned sexual likes and dislikes can be persistent. In addition, the present study also revealed some remarkable gender differences in aversive conditioned sexual responding.

In accordance with the expectations, women's genital blood flow in the extinction phase was attenuated in response to the erotic picture that was previously paired with the painful electric stimulus (the CS+) as compared to the erotic picture that was never paired with the US (the CS-). However, no such conditioned response in men was detected. Contrary to the expectations, men did not demonstrate a difference in penile circumference in response to the picture that was followed by painful stimulation during the acquisition phase. In fact, men displayed a slight increase in penile circumference over trails during the acquisition phase in response to the two CSs, even though the CS+ was followed by the painful stimulation. In addition, it was expected that the pairing of the erotic picture with painful stimulation would result in lower sexual arousability ratings of this picture. However, only women rated the CS+ as slightly less sexually arousing compared to the erotic picture that was never followed by painful stimulation, while men did not demonstrate attenuated subjective sexual response in response to the CS+ during the first trials of the extinction phase.

Crucial to the hypothesis, subjective affective value was successfully modulated by repeated association of the erotic stimulus with pain. Men and women rated the erotic picture that was paired with pain stimulation during the acquisition phase as more negative than the erotic picture that was not paired with pain. Remarkably, this conditioned differential responding lasted 10 extinction trials. Thus, classical conditioning using an erotic CS and an aversive pain US results in a conditioned response not only at the physiological level in women, but also at the subjective affective level in men and women. The present study demonstrated that although the attenuated genital response in men was absent, the more negative evaluation of the erotic stimulus seemed to remain over quite a number of extinction trials in men and women. However, crucial to the hypothesis and not corroborating earlier research (Vansteenwegen et al., 2006), in the present study the difference in affective evaluation of the

CS+ and the CS did decrease over time during the extinction phase, suggesting that conditioned sexual likes and dislikes are not entirely resistant to extinction.

In the present study, the habituation to the US and the nature of the CSs may have been potentially important confounding variables. First, after repeated exposure to the US, it is most likely that the US itself became less aversive leading to smaller conditioned affective value. Although a majority of the participants rated the US as highly unpleasant directly after the level of the pain stimulus was determined, in the exit interview afterwards the majority of participants reported that they perceived the pain stimulus as only moderately painful. Thus, although it may safely be concluded that the pain stimulus elicited an unconditional aversive emotional response, it should also be mentioned that the intensity of the US was only moderate.

Nevertheless, the finding that aversively conditioned sexual responses to sexual stimuli are affected by an extinction procedure whereas aversively conditioned responses to neutral pictures are not (Vansteenwegen et al., 2006) suggests a difference in the underlying learning processes. Research has shown that resistance to extinction in animal related and socially related fears (Mallan, Sax & Lipp, 2009; Rowles, Lipp & Mallan, 2012) is mediated by different evolutionary learning systems (Öhman, 1986). Like the proposed concept of evolved fear modules by Öhman and colleagues (Mineka & Öhman, 2002; Öhman & Mineka, 2001), it seems plausible that sexual arousal can also have a phylogenetic or evolutionary basis for sexual arousal-relevant selective associations. Indeed, it has been hypothesized that humans are born with sensitivity to what we call sexual stimuli and may be prepared to form particular associations between stimuli and sexual arousal (Everaerd, Laan & Spiering, 2000; Janssen et al., 2000). A characteristic of this proposed sexual arousal module is its tendency to be preferentially automatically activated by sex-relevant stimuli. Like the fear module, this sexual arousal module is relatively independent of higher cognitive influences such as expectancies. Results from

animal research suggest that conditioned responses toward sexually relevant CSs might be highly resistant to extinction (Domjan, O'Vary & Greene, 1988). This has led to the assumption that CS-US similarity is an important factor in conditioning (Krause, Cusato & Domjan, 2003; Rescorla & Furrow, 1977). It is thought that those 'prepared' associations are acquired more easily and that, additionally, these associations are thought to obey different laws of learning than nonprepared associations do. Derived from this, one could speculate that in combination with the independency of higher cognitive influences such as expectancies on the module of sexual arousal, the association between sexual stimuli and the suppression of sexual arousal (i.e. CS-US dissimilarity) like in the present study, may therefore not be straightforward, especially in healthy sexually functional men and women.

In addition, results from the Approach and Avoidance task did also not show clear resistance of extinction of evaluative learning. The pairing of the CS+ with the pain stimulus did not result in avoid tendencies towards this CS in men and women. Apparently the CS+ retained or regained enough sexual rewarding properties to even elicit approach tendencies in men and women. In addition, the introduction of new explicit erotic stimuli (i.e. the CS-alike stimuli) may have compromised the performance on this task, especially for men. Research has demonstrated that for men, more than for women, visual erotic stimuli preferentially recruit an amygdalo-hypothalamic pathway (Hamann et al., 2004). When limited in cognitive resources as a result of distraction and strong activation of reward structures, this plausibly could have led to difficulties in performance at the AAT. The newly introduced appetitive and biologically salient stimuli may have resulted in a generalized approach bias towards all stimuli, not only in men, but also in women. Therefore, in further research, distraction has to be limited by restricting the content of stimuli to only CS+ and CS- stimuli and eventual neutral pictures.

The results from the present study support the view that in women aversive sexual experiences, like in dyspareunia or in sexual assault, may result in decreased sexual arousal as a result of associative learning (van Berlo & Ensink, 2000; Both et al., 2008a; Brauer et al., 2007). However, the results from the present study do not corroborate the limited evidence in men for suppression of erectile responses through pairing of erotic stimuli with painful stimulation (Brom et al., 2014a). Important to note and mentioned before, is that studies providing such evidence were poorly controlled. Yet, the results concerning male genital measurements and sexual arousability ratings of the erotic picture in the extinction phase corroborates with results from animal studies (Barfield & Sachs, 1968; Caggiula & Eibergen, 1969; Crowley, Popolow & Ward, 1973; Farmer et al., 2014). First, in the present study conditioned attenuated subjective sexual arousal was demonstrated only in women. Intriguingly, in men, the pairing of the CS+ with the pain stimulus rendered that specific erotic picture subjectively more negative, but not less sexually arousing. Second, men did not demonstrate attenuated conditioned genital response in response to the CS+, but even demonstrated a trend for increased penile circumference during the acquisition phase. These results provide the first experimental evidence for sex differences in the disruption of sexual motivation by pain in humans. In women pain seems to diminish the rewarding properties of sexual activity and to adversely influence sexual motivation (Basson et al., 2010; Farmer et al., 2014; Fine, 2011). In contrast, male sexual behaviour seems to be unaffected by pain, or at least painful stimulation. Sexual pain disorders have been reported in 10% to 15% of women and less than 5% of men (Rosen et al., 2000), and are often accompanied by low sexual arousal, especially in women. Farmer et al. (2014) speculate that the pain induced reduction in sexual interest in female mice may be explained by the current incompatible physiological state with respect to a possible pregnancy. The results from the present study may indeed also be explained by this, as women,

just like female mice, have higher parental investment (Trivers, 1972). In contrast, also from an evolutionary perspective, the fittest individuals in a population are those that pass their genes on with the greatest frequency. Therefore male mice and men that copulate regardless of their or their partner's current circumstances, will increase the chances of producing offspring.

It could be that the general arousal response in men resulted in a spill-over effect in genital arousal in men in some way. Beach et al. (1956) administered shocks to rats in an attempt to inhibit mating tendencies. Interestingly, they found that while high levels of shocks did inhibit copulatory behaviour in male rats, low levels actually enhanced copulation. Results from those animal studies were explained in terms of excitement from the shock being summated with sexual stimulation to influence sexual arousal: the stimulation of mounting can be attributed to the increase in arousal caused by the shock. Results from the present study can be interpreted in a similar way. Second, as stated before, research (Hamann et al., 2004) has demonstrated that in men, visual erotic stimuli result in greater amygdalo-hypothalamic response as compared to women, making it plausible that the erotic pictures that were used as CSs in the present study were more rewarding for men than for women. Indeed, derived from analyses of the preconditioning phase and results from the exit interview, men rated the CSs as more positive and more sexually arousing and also declared afterwards to have liked watching the CSs more than women. This in combination with a moderate US, can potentially also explain why men did not demonstrate attenuated conditioned genital and subjective sexual response.

Women's subjective arousal is thought to appear to be largely reflective of how sexually exciting they find a stimulus and context (Everaerd et al., 2000; Laan, van Driel & van Lunsen, 2008), and genital engorgement does not reflect per se how women appreciate a sexual stimulus or context (Laan & Everaerd, 1995). In contrast, men's subjective sexual arousal is thought to correlate

closely with their degree of genital arousal. As studies in women with arousal disorders have demonstrated, they typically report no subjective arousal or minimal sexual arousal accompanied by negative emotions, but are found to display identical genital arousal reactions as compared to women who are acting as controls, and who are subjectively aroused by visual erotic stimulation (Everaerd et al., 2000; Laan van Driel & van Lunsen, 2008; van Lunsen & Laan, 2004).

For future studies it would be interesting to include a between subjects (unpaired) control group. With such a control group one can determine even more precisely whether and what learning has occurred. At present it is unclear if the decreased genital arousal towards the CS+ and CS- was due to conditioning or to pseudo conditioning (Hoffmann et al., 2014). Furthermore, in the present study, due to the use of different measures to assess genital response, direct comparison of genital response between men and women could not be made. Genital temperature reflects blood flow which is thought to be the physiological basis for sexual arousal, and has been shown to reliably increase with exposure to erotic stimuli and correlate significantly and highly with subjective reports of sexual arousal (Payne & Binik, 2006). Thermal imaging or devices such as labial thermistor clips and equivalent penile thermistor allow for comparison between the sexes. Therefore, it may be interesting for future studies to use other methodology like thermal imaging or genital thermistor clips. In addition, to increase ecological validity of conditioning studies, future research may use mild painful stimulation to the genitals (e.g. labia) as US. Furthermore, research has demonstrated that disgust may be an important aversive factor involved in sexual dysfunction in women (de Jong et al., 2010). In women with vaginismus (Genito-pelvic pain disorder/penetration disorder) sex related stimuli elicit disgust responses rather than sexual arousal (Borg, de Jong & Schultz, 2010). Therefore, it would be interesting for future studies to make use of an US with a disgusting nature.

Likewise, it is suggested that the amount of attention captured by sexual stimuli is a stronger predictor of an individual's sexual desire level than the valence of the emotional responses elicited by such stimuli (de Jong et al., 2010). Prause and colleagues (Prause, Janssen & Hetrick, 2008) demonstrated that participants with high levels of sexual desire were slower to detect targets in a dot detection task that replaced sexual images. Derived from this, it would be interesting for future studies on the conditioning of sexual response to also incorporate measures of attention.

The time frames SIR and TIR that were used for registration of genital response overlapped with the moment participants answered the questions regarding affective value and subjective sexual arousal. The answering of questions could have been distracting for participants, possibly negatively impacting genital response magnitude, especially in men. Research has shown that contiguously using a device such as a lever with processing of a sexual stimulus does not affect genital responding in women, whereas in men, it does result in lower genital responses (Chivers et al., 2010). However, notwithstanding any possible distraction, this possible distraction accounts for both CSs, making detection of differential responding clearly not impossible.

Results from the present study suggest that women are more sensitive to aversive sexual conditioning than men. However, it is unclear if this sensitivity has anything to do with experience in masturbation to erotic imagery, as this possibly may act as a kind of sensory preconditioning in men that overrides subsequent attempts at mild aversive conditioning. Furthermore, it is possible that the erotic pictures that were used as CSs in the present study were more rewarding for men than for women (Hamann et al., 2004). Future studies should investigate if stronger CRs can be obtained using erotic film clips as CSs. Although research has demonstrated that male sexual arousal can be conditioned making use of erotic explicit slides (Klucken et al., 2009; Plaud &

Martini, 1999), film clips generally elicit strong sexual response (Rupp & Wallen, 2008).

Results from the present study have some important implications for the treatment of sexual motivation problems in women. It seems beneficial to focus especially on subjective affect in the treatment of low sexual desire in women. As the present study has demonstrated, acquired sexual likes and dislikes can be persistent, but eventually do extinct. Therefore, in the treatment of sexual disorders with a learned component like low sexual desire, a combination of cue exposure and counterconditioning would be advisable. In counterconditioning, the CS is paired with a stimulus evoking a response that is incompatible with the original unconditioned response, thereby altering the valence of a stimulus (Baeyens et al., 1992). Research on appetitive conditioning has shown that counterconditioning is more effective than extinction alone in changing evaluations of the CS (van Gucht et al., 2010). In addition, as research has demonstrated that resilient women with a history of sexual trauma are more successful at cognitively enhancing emotional responses to aversive pictures as compared to women with PTSD after sexual trauma and even to healthy, non-traumatized controls (New et al., 2009), it is suggested that resilience is associated with the ability to sustain attention to unpleasant stimuli, leading to a more accurate or optimistic appraisal of the perceived threats. This makes clear that next to the technique of counter-conditioning, there is a role for cue-exposure therapy in the treatment of sexual arousal disorders with a learning history. And although extinction procedures do not seem to erase the originally learned CS-US association (Bouton & Moody, 2004; Brom et al., 2014b), CS-alone presentations may extinguish conditioned responses, as demonstrated. It is speculated that an extinction procedure makes the original CS-US associations less retrievable from memory, whereas it does enhance the accessibility of a new CS-no US association (Delemater, 2004). Therefore, depending on how strong and how easily available CS-US associations are, cue

exposure therapy seems relevant for the treatment of sexual disorders with a learned component, like hypo or hypersexuality. In addition, the finding that sexual conditioned responses extinguish dependent upon context (Brom et al., 2014b) make clear that extinction procedures may best be applied in the context in which the problematic behaviour is experienced, generalizing to other contexts and with multiple stimuli.

To conclude, in the present study genital and subjective sexual responses in women were successfully modulated by the aversive conditioning procedure, but intriguingly men did not demonstrate attenuated genital and subjective sexual response. The use of erotic pictures as CS (that are possibly more rewarding for men than for women) in combination with a moderate US, can potentially account for this. Results from the present study provide evidence that conditioned sexual likes and dislikes can be persistent, although conditioned affect eventually does extinguish. A combination of extinction and counter-conditioning (learning a new opposite response) would plausibly be more effective than extinction alone in the treatment of sexual arousal disorders with a learned component.

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Chapter 6

Extinction and Renewal of Conditioned Sexual Responses

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Abstract

Extinction involves an inhibitory form of new learning that is highly dependent on the context for expression. This is supported by phenomena such as renewal and spontaneous recovery, which may help explain the persistence of appetitive behavior, and related problems such as addictions. Research on these phenomena in the sexual domain is lacking, where it may help explain the persistence of learned sexual responses. Men (n=40) and women (n=62) participated in a differential conditioning paradigm, with genital vibrotactile stimulation as US and neutral pictures as conditional stimuli (CSs). Dependent variables were genital and subjective sexual arousal, affect, US expectancy, and approach and avoid tendencies towards the CSs. Extinction and renewal of conditioned sexual responses were studied by context manipulation (AAA vs. ABA condition). No renewal effect of genital conditioned responding could be detected, but an obvious recovery of US expectancy following a context change after extinction (ABA) was demonstrated. Additionally, women demonstrated recovery of subjective affect and subjective sexual arousal. Participants in the ABA demonstrated more approach biases towards stimuli. The findings support the context dependency of extinction and renewal of conditioned sexual responses in humans. This knowledge may have implications for the treatment of disturbances in sexual appetitive responses such as hypo- and hypersexuality.

6.1. Introduction

It is thought that contexts play an important role in regulating responses and in related relapse behavior (Bouton, 2002; Thewissen, Snijders, Havermans, van den Hout & Jansen, 2006). The role of context is best exemplified by the phenomenon of renewal. Renewal is the term used to describe recovery of extinguished behavior as a result of context change (Bouton & Moody, 2004). The renewal phenomenon has been demonstrated for Pavlovian and instrumental responding based on numerous reinforcers, including natural rewards such as food (Nakajima et al., 2000) and drug rewards (Crombag, Grimm & Shaham, 2002). Unfortunately, given its relevance for extinction-based treatments, studies on extinction and renewal in the human sexual domain are completely lacking. In the present paper, we report an experiment on extinction and renewal of conditioned sexual responses in sexually functional men and women.

According to incentive motivation models, sexual motivation is the result of the interplay of a sensitive internal sexual system with motivational stimuli. Stimuli that can promote motivation are called incentive stimuli (Bindra, 1974; Singer & Toates, 1987). Their motivational valence can be unconditioned or conditioned as a result of associative leaning (Di Chiara, 1995). Some stimuli (e.g. genital touch) may be innately sexually competent (i.e. stimuli that can elicit sexual response without a learning history) and can therefore serve as incentive stimuli, but many sexual stimuli are not intrinsically sexually competent. Specific cues of sexually competent stimuli may gain learned incentive value through their association with the stimulus. Several studies, including some from our lab, have demonstrated conditioned sexual arousal responses in humans (for a review see Brom, Both, Laan, Everaerd & Spinhoven, 2014). In classical conditioning, through the repeated association of a neutral stimulus (NS) with the unconditional stimulus (US), the NS will eventually trigger the same reaction as the US. Since the NS is no longer

ineffective in eliciting a response but has become a conditioned stimulus (CS), the reaction to the CS is called the conditioned response (CR) (Bindra, 1974; Pavlov, 1927). Subsequent repeated presentations of a CS without the US will result in a loss of conditioned responding, as the CS no longer predicts the aversive or appetitive US (Delamater, 2004). This learning process is known as *extinction* and has obvious clinical relevance as it is thought to be the core mechanism for exposure therapy (Hermans, Craske, Mineka & Lovibond, 2006; Myers, Carlezon and Davis, 2011; Rescorla, 2001). In exposure therapy, conditioned responses are lessened or inhibited by repeated or prolonged exposure to a cue (the CS) in absence of the event it used to predict (the US). However, many individuals relapse after being 'cured'. Therefore, although CS-alone presentations may extinguish conditioned responses, the extinction procedure does not seem to erase the originally learned CS-US association. It appears that this original association is retained (Bouton & Moody, 2004).

Conditioned responding can 'renew' following a context shift out of the extinction context (Bouton, 2002). Renewal is the restoration of the CR in context A but not in context B when learning occurred in context A and extinction in context B. Extrapolating the renewal phenomenon to clinical practice, someone who acquired craving for internet-sex at home (context A), and is successfully extinguished by cue exposure therapy in a therapeutic setting (context B), may experience strong craving upon changing context such as sitting behind the computer at home (context A).

The assumption that conditioned responses extinguish dependent upon context has been supported by animal studies (for a review see Bouton, 2004). In humans, the phenomenon of renewal is mainly studied in fear paradigms or studies on addiction (Effting & Kindt, 2007; Kalisch et al., 2006; Stasiewicz, Brandon & Bradizza, 2007; Thewissen, Snijders, Havermans, van den Hout & Jansen, 2006). It is demonstrated that fear returns when individuals are tested in a context different from the treatment context (Hermans, Craske, Mineka &

Lovibond, 2006). In a differential fear conditioning experiment by Vansteenwegen et al. (2005), a neutral slide of a pictorial face (CS+) was paired with a loud noise (US). The CS+ is the stimulus that is being followed by the US, whereas the CS- is not. Extinction of conditioned fear was established by presenting the CS without the US in a different context. Different contexts were obtained by manipulating the lighting in the experimental room, and acquisition took place in either a dark or illuminated room. When returning to the original acquisition context (i.e., ABA renewal), conditioned fear responding to the CS+ renewed. Effting and Kindt (2007) replicated this renewal effect in humans within an ABA renewal paradigm as used by Vansteenwegen et al. (2005), making use of shocks as US. Changing the context after the extinction phase resulted in a significant increase of US expectancy ratings to CS+ relative to CS- in Context A. However, no robust renewal effect for electrodermal responses could be demonstrated. In addition, there is evidence for renewal of conditioned responses following a context change in appetitive conditioning (Van Gucht, Vansteenwegen, Beckers & Van den Bergh, 2008; Thewissen, Snijders, Havermans, van den Hout & Jansen, 2006).

Although the evidence regarding renewal in human learning has accumulated in recent years, studies on renewal of sexual conditioned responses are lacking, despite the possible important implications for exposure-based treatment strategies for learned maladaptive sexual responses. The finding that paraphilia, hypersexuality and related sexual disorders are predominantly observed in men (Kafka, 1994; Kuzma & Black, 2008) has led to the idea that men are more receptive to sexual conditioning than women, resulting in increased CR acquisition (Pfaus, Kippin & Centeno, 2001). However, at present, it is not clear if gender differences in sexual conditionability do exist as results of conditioning studies are mixed (Hoffmann, Janssen and Turner, 2004; Klucken et al., 2009, 2013). However, a vast amount of research has shown that the neurotransmitter dopamine plays a major role in associative learning and

sexual behaviour (Berridge, 1996; Oei, Rombouts, Soeter, Gerven, & Both, 2012; Schultz, 2006), and as gender differences in the number of dopamine neurons are influenced by several factors, including sex chromosome complement (Lombardo et al., 2012), the presence of the Sry gene (Dewing et al., 2006) and gonadal hormones, it is therefore thinkable that differences in sexual conditionability do exist between men and women, with men being more receptive to sexual conditioning. This, combined with the fact that adolescent maturation is a sensitive period for steroid dependent organization of neural circuits involved in sexual stimulus salience, sensory associations and sexual motivation (Sisk & Foster, 2004), and the finding that for men, more than for women, visual stimuli preferentially recruit an amygdalo-hypothalamic pathway (Hamann, Herman, Nolan & Wallen, 2004), gender differences in sexual conditionability seem plausible. In addition, it is proposed that sexual preferences of men are greatly influenced by early learning experiences, particularly during defined critical periods, such as adolescence (Coria-Avila, 2012; Pfaus, Kippin & Centeno, 2001). In addition, women are believed to have more 'erotic plasticity' (Baumeister, 2000). The contradictory results of previous studies point to the importance for further investigation of possible gender differences in sexual learning.

The present study is the first to investigate extinction and renewal of conditioned sexual responses in men and women. The experimental procedure of Effting and Kindt (2007) was closely followed, except that now a sexually pleasurable tactile stimulus (vibrotactile genital stimulation) served as US. Two neutral pictures served as CSs, and subjective affect, subjective sexual arousal, US expectancy ratings and genital arousal were dependent variables. It was predicted that participants in both conditions (AAA and ABA) would show conditioned sexual responding after acquisition trials, which was expected to gradually decrease. As an index of renewal, it was predicted that upon a context change after extinction, the ABA condition would show recovery of

conditioned responding on the test trials as compared to the last extinction trial. No increases were expected during these test trials in the AAA condition. In addition a stimulus response compatibility task (Approach and Avoidance Task, AAT) was included to assess implicit approach and avoidance tendencies towards the CS (Chen & Bargh, 1999). It was predicted that upon a context change after extinction, participants in the ABA condition would show stronger approach responding to CS+ relative to CS− on the AAT as compared to participants within the AAA condition.

6.2. Method

6.2.1. Participants

Written consent was obtained from all participants before participation. Research participants were 40 men and 62 women. Participants were paid (€30,-) or received course credit for their participation. Participants were recruited through advertisements on social networks, and at the Universities of Leiden and Amsterdam. Inclusion criteria were: age between 18 - 45 years and a heterosexual orientation. Exclusion criteria were: sexual problems, an affective or psychotic disorder or abusive drug use, pregnancy or breastfeeding, and a medical illness or medication use that could interfere with sexual response. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center.

6.2.2. Design and conditioning procedure

The experimental design involved differential conditioning with one stimulus (the CS+) being followed by genital vibrostimulation (US) during the acquisition phase, whereas the other stimulus (CS-) was never followed by

genital vibrostimulation. Which of the two stimuli served as the CS+ was counterbalanced across participants and conditions. In the ABA condition, participants received acquisition in one context (Context A), extinction in another context (Context B), and a test for renewal in the original acquisition context (Context A). In the AAA condition, acquisition, extinction, and testing took place in one and the same context (Context A). The colors of the lighting that served as Contexts A and B were counterbalanced across participants. For a schematic overview of the procedure see Figure 1.

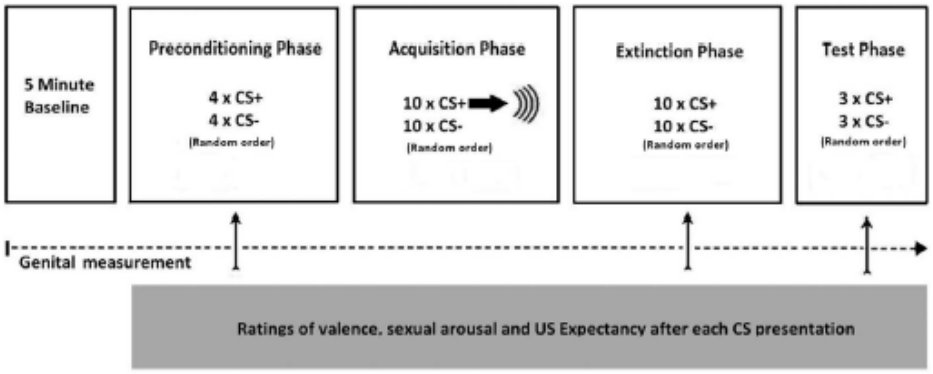

Figure 1. Schematic representation of the experimental procedure in both context conditions. In the AAA-condition, acquisition phase, extinction phase and test phase were in the same lighting context. In the ABA-condition the extinction phase was in a different lighting context than the acquisition phase and test phase.

In the preconditioning phase, participants saw four nonreinforced presentations of the CS+ and four presentations of the CS-. Subsequently, in the acquisition phase the contingency between CS+ and US was learned. During this phase both the CS+ and CS- were presented 10 times each and the CS+ was always followed by the US. For participants in all conditions acquisition took place in Context A. The extinction phase consisted of 10 unreinforced CS+ presentations and 10 unreinforced CS- presentations. After

the extinction phase a test phase of 3 unreinforced CS+ presentations and 3 unreinforced CS- presentations was presented. During the whole procedure inter-trial intervals (ITIs) were 20, 25, or 30s. The order of the length of the ITI was random, with the restriction of only two successive lengths. For participants in the AAA condition, extinction occurred in Context A, while for participants in the ABA condition extinction took place in a different context (Context B). The basic design for testing conditioning effects was a 2 (CS+ vs. CS-) x 10 (trial) within subjects design. Similarly, the basic design for testing renewal effects was a 2 (CS+ vs. CS-) X 3 (trial) within subjects design.

6.2.3. Materials, Apparatus, and Recording

Stimulus Materials Two neutral pictures served as CS+ and CS-. Each picture portrayed a black and white cartoon-like drawing of the head of a person. During intervals between the pictures, a white screen was presented. The CS+ and CS- were presented for 9s each.

Genital vibrostimulation (US) was provided only during the acquisition phase, 8s following the start of each CS+ for 2s. For male participants, the vibrotactile genital stimulation was administered by means of a hands-off ring-shaped vibrator (Aquasilks), which was placed by the participants themselves just below the coronal ridge. For women, a small hands-off vibrator (2 cm diameter) was used, placed on the clitoris. The participants were instructed to place the vibrator in such a way it was *most sexually stimulating*.

Context manipulation Contexts were manipulated by illuminating the experimental room in either a pink or a yellow light. Lighting was supplied by a frame with six fluorescent tubes of 36 W (two pink and four yellow tubes), resulting in a

slightly dimmed colored illumination of the whole room. The experimenter controlled the lighting from an adjacent room.

Male genital sexual arousal was measured using an indium/gallium-in-rubber penile gauge assessing changes in circumference of the penis (Bancroft, Jones, & Pullen, 1966). The penile gauges were calibrated before each laboratory session using a set of calibrated rings (Janssen, Prause, & Geer, 2006). Participants were instructed to place the gauge midway along the penile shaft. Changes in electrical output caused by expansion of the gauge were recorded by a continuous DC signal. The indium/gallium penile gauges were disinfected after each use, according to Sekusept plus disinfection procedure. Sekusept plus contains Glucoprotamine, which action spectrum covers bacteria including mycobacteria, fungi and viruses (e.g. Human Papillomavirus [HPV]) (MedCaT B.V.).

Women's genital arousal was measured using a vaginal photoplethysmograph assessing vaginal pulse amplitude (VPA) (Laan, Everaerd & Evers, 1995). The photoplethysmograph is a menstrual tampon-sized device containing an orange-red light source and a photocell. The light source illuminates the capillary bed of the vaginal wall and the blood circulation within it. The photoplethysmograph was disinfected at the LUMC by means of a plasma sterilization procedure between uses. Plasma sterilization is a highly effective method for the complete removal of all organic (and certain in-organic) material. Genital response was measured continuously during resting baseline, preconditioning, acquisition, extinction, and test phases.

Subjective Ratings Ratings of affective value, sexual arousal and US expectancy were collected during the preconditioning-, extinction- and test phase. Participants were first asked to rate, after each CS presentation, the affective

value of the CSs by answering the question "*What kind of feeling does this picture evoke in you*?" on a seven-point Likert scale on a keyboard that varied from *very negative* to *very positive*. Then, subjective sexual arousal was rated by answering the question "*How sexually arousing is this picture to you*?" on a seven-point scale that varied from *not sexually arousing at all* to *very sexually arousing*. Then, US expectancy was rated by answering the question "*To what extent did you expect a vibration after this picture*"? on a seven-point scale labeled from '*certainly no vibration'* to '*certainly a vibration'*.

Approach Avoidance Task (AAT, Cousijn, Goudriaan & Wiers, 2011). This task assesses approach and avoidance motivational processes by requiring participants to respond to irrelevant feature of pictures by either pulling a joystick handle toward them or by pushing it away. The amount of time required to execute these actions is the dependent variable. Participants were presented with the CS+ and CS- pictures from the experiment, as well as neutral pictorial objects and cartoon faces resembling the CSs. Literature supports the AAT's validity in measuring approach/avoidance motivational processes (Wiers, Rinck, Kordts, Houben, & Strack, 2010).

6.2.4. Procedure

Each participant was tested individually by a trained experimenter of the same sex. After participants had given informed consent, the experimenter explained the experimental procedure and the use of the plethysmograph, penile gauge and vibrator. Then, the experimenter left the room to allow the participant to place the genital devices privately. Further instructions were given through written instructions on the monitor. Then a 5-minute resting period followed, during which a neutral film was played and baseline measurements of genital response were collected during the last 2 minutes. Then the preconditioning, acquisition, extinction and test phases followed. Immediately after the

experimental procedure and after the participant removed the genital devices, the AAT was presented in the experimental room with the same lighting conditions as in the original acquisition context (A). After completing the AAT, participants filled in questionnaires about demographics, and sexual function (e.g., FSFI, IIEF). Finally, an exit interview questionnaire was administered.

6.2.5. Data Reduction, Scoring and Analysis

A software program (VSRRP98; developed by the Technical Support Department of Psychology, University of Amsterdam) was used to analyze the genital data. Mean penile circumference or mean VPA level during the 2-minute resting baseline period was calculated. Genital responses to the CSs were scored in three latency windows: during 4-8, 9-12 and 13-16 s following CS onset, respectively FIR (first interval response), SIR (second interval response) and TIR (third interval response). For FIR, SIR and TIR, change scores were calculated for each CS presentation by subtracting mean genital resting baseline from genital measures following CS presentation. For genital responses, effects were tested separately for men and women, with Mixed ANOVA's (General Linear Model in SPSS), with Stimulus and Trial as within-subject factors and Condition as between subjects factor. Analyses of subjective measurements and AAT scores were conducted for men and women combined. For subjective ratings, effects were tested with Mixed ANOVA, with Stimulus and Trial as within-subject factors and Condition and Gender as between subjects factor. The Greenhouse–Geisser correction was applied to adjust for violation of the sphericity assumption in testing repeated measures effects. Preconditioning, acquisition, extinction, and test phases were analyzed separately. Effect sizes are reported as proportion of partial variance (η_p^2) (Cohen, 1988). For the AAT, bias scores were calculated by subtracting median approach RT from median avoid RT for each image category. Median RTs were used because they are less

sensitive to outliers than means. A positive bias score indicated a relatively faster approach compared to avoid RTs, whereas a negative score indicated a relatively faster avoid compared to approach RTs. To compare the AAA and ABA condition, bias scores were analyzed using ANOVA.

6.3. Results

Of the 62 women tested, genital data of 2 participants were left out. One data point in the acquisition phase of a male participant was discarded as outlier because this measure was above 4 SD from the mean (inclusion of this data point did not change results). Results from the AAT are based on 99 participants. Participants were randomly assigned to one of the two context conditions with the restriction that conditions were matched on sex as close as possible: AAA (N= 49; Men, n = 20) and ABA (N= 53; Men, n = 20). Men and women did not differ in age, in sexual functioning, nor in prior experience with vibrostimulation across conditions, see Table 1 *Subject characteristics*.

Variable	Men					Women					Men & Women				
	AAA (n= 20)		ABA (n=20)			AAA (n= 29)		ABA (n= 33)			Men (N=40)		Women (N= 62)		
	M	SD	M	SD	*p*	M	SD	M	SD	*p*	M	SD	M	SD	*p*
Age (years)	22.3	2.6	24.9	6.5	*.11*	21.5	2.8	22.5	2.9	*.60*	23.6	5.0	22	2.8	*.04*
Sexual Functioning (IIEF/FSFI score)	33.5	5.5	35.8	6.2	*.21*	26.6	2.4	26.4	2.9	*.83*					
Prior Experience Vibrostimulation	1.8	1	1.7	1	*.75*	3	1.3	2.9	1.2	*.85*	1.7	1	3.0	1.3	< *.01*
Pleasantness US	3.4	1.1	3.2	0.7	*.62*	3.4	0.9	3.3	0.8	*.53*	3.3	0.9	3.3	0.8	*.72*
US Perceived as Sexually Arousing	3.1	1.1	2.7	0.7	*.19*	3.1	0.9	3.1	0.8	*.41*	2.9	0.9	3.1	0.9	*.18*
Declared Sexual Arousal	2.4	0.9	2.1	0.7	*.30*	2.6	0.8	2.5	0.8	*.79*	2.2	0.8	2.6	0.8	*.06*

Table 1. Subject characteristics. Descriptive subject variables for men and women, and for each condition. Notes: Scale Prior experience vibrostimulation: 1 (never) – 5 (very often); Scale Pleasantness US: 1 (not pleasant at all) - 5 (very pleasant); Scale US perceived as sexually arousing: 1 (not sexually arousing at all) – 5 (very sexually arousing); Scale Declared sexual arousal: 1 (not sexually aroused) – 5 (very sexually aroused) ; * $p < .05$. Fourteen women indicated not to have a stable heterosexual relationship at the time of the study, and six women indicated not having had sexual activity with a partner during the last weeks, hence resulting in a low FSFI score.

6.3.1. Genital Sexual Arousal

Preconditioning phase

Responses for three latency windows were analyzed: first, second, and third interval response (FIR, SIR, TIR). Analyses were conducted to verify equal levels of penile circumference and VPA in response to the CSs during the preconditioning phase. For FIR and SIR, no difference in circumference or VPA following presentation of the CS+ and CS- was found, *p*s > .11. VPA TIR in response to the CS- was higher as compared to the CS+, although this difference did not reach conventional level of significance, VPA, $p < .08$, $\eta_p^2 = .06$.

Acquisition phase

Men Figure 2a summarizes penile circumference (TIR) to CS+ and CS- across trials for both conditions separately. The analysis of penile circumference in the acquisition phase revealed a main effect of Stimulus, FIR, $F(1, 36) = 12.39$, $p < .01$, $\eta_p^2 = .26$, SIR, $F(1, 35) = 83.68$, $p < .01$, $\eta_p^2 = .70$, TIR, $F(1,35) = 16.96$, $p < .01$, $\eta_p^2 = .33$, meaning the vibrostimulation resulted in a genital response, as can be seen in Figure 2a. Contrary to the expectation, penile circumference to CS- was larger as compared to CS+. No effects for Trial were observed, all *p*s> .24, and no significant 2 (Stimulus) X 10 (Trial) interaction was found, all *p*s> .39. This pattern of acquisition did not differ between conditions as reflected by non-significant 2 (Stimulus) X 10 (Trial) X 2 (Condition) and 2 (Stimulus) X 2 (Condition) interactions, all *p*s>.41.

Women In line with previous studies (Both et al., 2008; Both, Brauer & Laan, 2011), the analyses of VPA during the acquisition phase did not reveal a main effect of Stimulus on FIR, $p < .08$, and SIR, $p = .28$, whereas it did on

TIR, showing that VPA was higher following the presentation of the CS+ plus vibrostimulation than following the CS-, $F(1, 56) = 27.74$, $p < .01$, $\eta_p^2 = .33$. As can be seen in Figure 2b, the vibrostimulation resulted in a genital arousal response. No significant effects for Trial were observed, FIR, $p = .53$; SIR $p = .07$; TIR $p = .15$. However, an interaction effect of Stimulus and Trial for VPA TIR was found, $F(5, 268) = 6.73$, $p < .01$, $\eta_p^2 = .11$, indicating differentiation between genital responding to CS+ plus vibrostimulation and CS- over trials. This pattern of acquisition did not differ between conditions as reflected by a non-significant 2 (Stimulus) X 10 (Trial) X 2 (Condition) effect, $p = .85$.

Extinction phase

Men The 2 (Stimulus) X 10 (Trial) X 2 (Condition) Mixed ANOVA revealed no overall larger penile responses to CS+ than to CS-, and showed no significant Stimulus X Trial interaction, *p*s> .17. This indicates that there was no difference in penile responding towards the CS+ and CS-, and this pattern of responding did not change across extinction trials. In addition, no differences between the conditions were seen, *p*s> .30. An additional 2 (Stimulus) X 2 (Phase; Mean trial 1-4 precon and the first extinction trial) Mixed ANOVA for penile circumference, revealed a trend for Stimulus on FIR, $F(1, 37) = 2.92$, $p < .10$, SIR, $F(1, 37) = 2.85$, $p = .10$, and TIR, $F(1, 37) = 2.99$, $p = .09$. However, no interaction effect for Stimulus and Trial was observed, *p*s> .80, indicating no conditioned differential responding on the first extinction trial. Analysis of the entire extinction phase yielded no main effect for Trial, FIR $p = .23$; SIR $p = .23$; TIR $p = .23$. The additional 2 (Stimulus) X 2 (Phase; Mean trial 1-4 precon and the last extinction trial) Mixed ANOVA revealed no main effect for Stimulus, *p*s> .58. In summary, the picture that was reinforced by genital vibrostimulation during the acquisition phase did not elicit greater penile circumference during

the extinction phase, and both conditions did not differ in genital responding to the CSs, see Figure 2a.

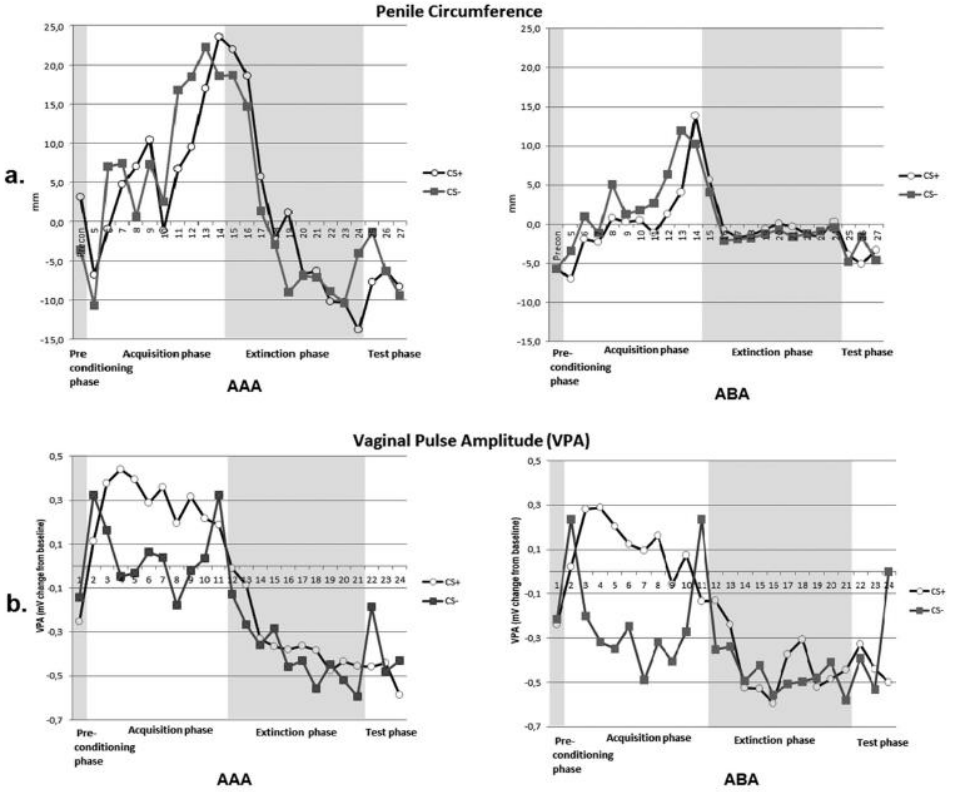

Figure 2. Mean penile circumference change scores (a.) and Mean vaginal pulse amplitude (VPA) change scores (b.) during the third interval response window (TIR) following the CS+ and CS- during the preconditioning phase, acquisition phase, extinction phase and test phase for the two conditions AAA and ABA. Note that during the acquisition phase, the response represents responding to the CS+ plus the US.

Women Because extinction of conditioned responding cannot be expected when there is no acquisition of conditioned responding, VPA FIR results are not reported. As expected a significant main effect for Stimulus was found, SIR, $F(1, 57)= 4.73$, $p< .03$, $\eta_p^2 = .04$; TIR, $F(1, 56)= 5.78$, $p= .02$, $\eta_p^2 = .09$, meaning

the picture that was reinforced by clitoral vibrostimulation during the acquisition phase did elicit higher VPA during the extinction phase, as can be seen in Figure 2b. Most crucial to our hypothesis, the ANOVA showed no significant interaction effect between Stimulus and Trial, SIR, p= .21, TIR, p=.21, meaning no extinction effect. The analysis also revealed that this pattern of differential responding towards CS+ and CS- did not differ between conditions, SIR, p= .30, TIR, p= .91. As expected, additional analysis of the first extinction trial yielded significant differences for VPA SIR $F(1, 57)= 7.74$, $p< .01$, $\eta_p^2 = .12$, and TIR, $F(1, 58)= 3.96$, $p= .05$, $\eta_p^2 = .06$. Also, the analysis of the last extinction trial yielded significantly higher VPA in response to the CS+ than in response to the CS- for VPA SIR, $F(1, 57) = 4.31$, $p= .04$, whereas no difference in VPA TIR could be detected, $p= .12$. Again, the pattern of differential responding towards CS+ and CS- did not differ between conditions, first extinction trial: ps> .24, last extinction trial: ps> .41. However, there was a main effect of Trial, indicating VPA was decreasing over time, SIR, $F(4, 228)= 3.66$, $p< .01$, $\eta_p^2 =.06$; TIR, $F(4, 215)= 3.88$, $p< .01$, $\eta_p^2 =.07$. In summary, the conditions did not differ in conditioned responding during the extinction phase: AAA and ABA showed an equal differential VPA responding to the picture that was reinforced by clitoral vibrostimulation during the acquisition phase, and for both conditions this differential responding showed no complete extinction across trials. However, for both conditions VPA was decreasing over time (see Figure 2b).

Test phase

Because recovery of conditioned responding cannot be expected when there is no acquisition of conditioned responding, results for men were not reported for the sake of brevity. For the same reason, VPA FIR results were not reported.

Women The analysis of main interest, the 2 (Stimulus) X 2 (Phase; last extinction trial and first test trial) 2 X (Condition) Mixed ANOVA, yielded a trend for Stimulus X Trial X Condition, $F(1, 56) = 3.10$, $p = .08$, $\eta_p^2 = .05$. The 2 (Stimulus) X 3 (Trial) X 2 (Condition) analysis of the test phase for VPA SIR, yielded borderline significance on VPA SIR, $F(1, 102) = 3.09$, $p < .06$. Inspection of Figure 2b suggests these effects may be explained by unexpectedly large responses to the CS-. Therefore, we additionally conducted a separate 2 (Phase) X 2 (Condition) ANOVA for only CS+ responses on the last extinction trial and first test trial (see also Effting & Kindt, 2007; Vansteenwegen et al., 2005). However, no significant interaction effect was seen for Stimulus X Condition, $p = .19$. For VPA TIR the interaction most crucial to our hypothesis, Stimulus X Phase X Condition, yielded no significance, $p = .19$. The analysis of VPA TIR on the last extinction trial and first test trial, yielded a main effect for Stimulus, $F(1, 56) = 4.18$, $p < .05$, $\eta_p^2 = .07$. Contrary to the expectations, no interaction effect for Stimulus X Phase X Condition was found, $p = .24$. Additional analysis of only CS+ responses during TIR, yielded no significance, $p = .39$. Hence, women showed no increased conditioned genital responding to the CS+ upon changing the context after extinction.

6.3.2. Subjective measures

Preconditioning phase

For US expectancy and affective value, no difference in responding to the CSs was found between conditions and between men and women, all ps > .20. However, for subjective sexual arousal there were marginally significant interaction effects for Stimulus X Gender, $p < .09$, and Stimulus X Condition X Gender, $p = .06$, meaning men and women tended to differ in ratings of

subjective sexual arousal towards the CSs, with men rating the CS+, and women rating the CS- as slightly more arousing.

Extinction phase

US Expectancy As can be seen in Figure 3, both conditions showed a robust increase of differential responding towards CS+ vs. CS- after the acquisition phase, and a decrease in this differential responding over trials. Analysis of US expectancy ratings during the preconditioning phase (Mean trial 1-4) and the first extinction trial, revealed a main effect for Stimulus, $F(1, 97)= 128.07$, $p< .01$, $\eta_p^2 = .57$, and an interaction effect for Stimulus and Trial, $F(1, 97)= 133.49$, $p< .01$, $\eta_p^2 = .58$, indicating a conditioning effect. The 2 (Stimulus) X 10 (Trial) X 2 (Condition) X 2 (Gender) Mixed ANOVA of the extinction phase yielded a significant Stimulus X Trial interaction, $F(3, 283)= 47.39$, $p< .01$, $\eta_p^2 = .34$. No significant Stimulus X Trial X Condition interaction was found, $p= .16$, meaning both conditions showed an equal loss of expecting the US after presentation of the CS+. Analysis of expectancy ratings on the first extinction trial and the last extinction trial, revealed a main effect for Stimulus, $F(1, 97)= 135. 09$, $p< .01$, $\eta_p^2 = .58$, and an interaction effect for Stimulus X Trial, $F(1, 97)= 118.95$, $p< .01$, $\eta_p^2 = .55$, indicating extinction of conditioned responding. Also a trend was detected for Stimulus X Trial X Condition, $F(1, 97)= 2.97$, $p< .09$, with the AAA condition showing stronger loss of US expectancy. Analysis of the first extinction trial yielded a significant main effect for Stimulus, $F(1, 97)= 147.36$, $p< .01$, $\eta_p^2 =.60$. Likewise, analysis of the last extinction trial also yielded a significant main effect for Stimulus, $F(1, 95)= 9.61$, $p< .01$, $\eta_p^2 = .09$, but also an interaction effect for Stimulus X Condition, $F(1, 95)= 4.02$, $p< .05$,

η_p^2 = .04. This indicates there was still a difference in the ABA condition in US expectancy in response to the CS+ and the CS- on the last extinction trial. In sum, men and women showed an equal loss of expecting the US after presentation of the CS+.

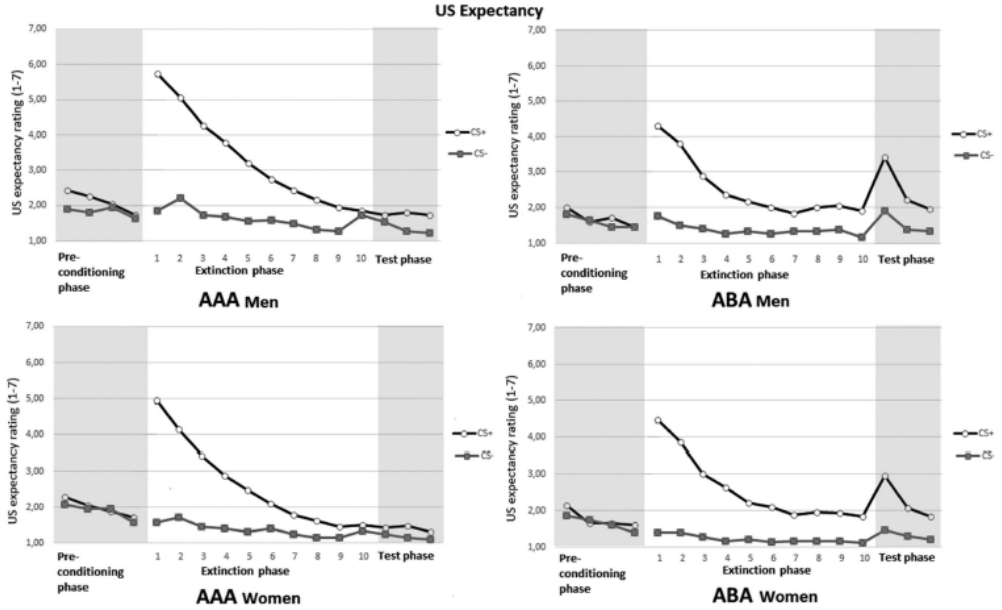

Figure 3. US expectancy ratings following the CS+ and CS- during the preconditioning phase, extinction phase and test phase for men (top) and women (bottom) in the two conditions AAA and ABA.

Affective Value Men and women differed in conditioned responding after the acquisition phase, see Figure 4. For women, both conditions showed a more robust increase of differential responding towards CS+ vs. CS- after the acquisition phase, and a decrease in this differential responding over trials. The 2 (Stimulus) X 2 (Phase; Mean Precon trial 1-4 and first extinction trial) X 2 (Condition) X 2 (Gender) Mixed ANOVA of the affective value ratings

revealed an interaction effect for Stimulus X Trial, $F(1, 97) = 29.73$, $p < .01$, $\eta_p^2 = .24$. Also an interaction effect was found for Stimulus X Phase X Gender, $F(1, 97) = 16.95$, $p < .01$, $\eta_p^2 = .15$. Analyses of the preconditioning phase and first extinction trial for men and women separately, yielded no significant interaction for Stimulus X Phase for men, $F(1, 38) = 1.59$, $p = .22$. This indicates there was no conditioned responding on subjective affect for men, as can be seen in Figure 4. For women, this analysis yielded a significant interaction effect for Stimulus X Phase, $F(1, 59) = 52.92$, $p < .01$, $\eta_p^2 = .47$. As expected, analysis of the extinction phase showed a significant Stimulus X Trial interaction, $F(4, 378) = 8.92$, $p < .01$, $\eta_p^2 = .09$, indicating that the difference in rated subjective affect between CS+ and CS- gradually decreased across trials, which constitutes extinction. The ANOVA yielded no Stimulus X Trial X Condition interaction $F(4, 378) = 0.62$, $p = .65$, but did yield a significant Stimulus X Trial X Gender interaction, $F(4, 378) = 7.52$, $p < .01$, $\eta_p^2 = .07$. Additional analysis of the first and the last extinction trial, revealed interaction effects for Stimulus and Trial, $F(1, 96) = 17.66$, $p < .01$, $\eta_p^2 = .16$, and Stimulus X Trial X Gender, $F(1, 96) = 14.37$, $p < .01$, $\eta_p^2 = .13$. No significant interaction effect for Stimulus X Trial X Condition was found, $p = .54$. Meaning, although both conditions showed equal loss of conditioned responding, this effect can be attributed to women's responding. Additional analyses of the first and the last extinction trial for men and women separately, revealed no interaction effect for Stimulus X Trial for men, $F(1, 37) = 0.10$, $p = .76$, meaning no extinction occurred. As expected, this analysis for women revealed a significant interaction effect for Stimulus X Trial, $F(1, 59) = 34.47$, $p < .01$, $\eta_p^2 = .37$, indicating extinction. Analysis of the first extinction trial yielded a significant main effect for Stimulus, $F(1, 97) = 28.19$,

p< .01, η_p^2 = .23, and significant interaction effect for Stimulus X Gender, F(1, 97)= 19.28, p< .01, η_p^2 = .17. Analysis of the last extinction trial still yielded a significant main effect for Stimulus, F(1, 97)= 5.69, p= .02, η_p^2 = .06, indicating differential responding towards the CS+ and CS-.

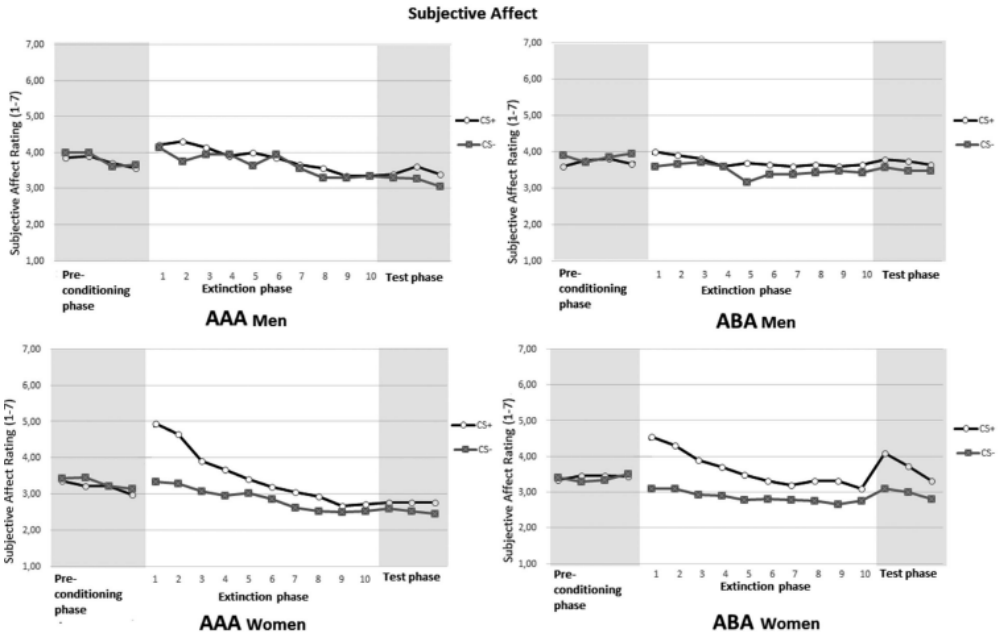

Figure 4. Subjective affect ratings following the CS+ and CS- during the preconditioning phase, extinction phase and test phase for men (top) and women (bottom) in the two conditions AAA and ABA.

Subjective Sexual Arousal Figure 5 shows increased ratings of sexual arousal towards the CS+ on the first trials of the extinction phase, which constitutes conditioned responding. The 2 (Stimulus) X 2 (Phase; Mean Precon trial 1-4 and first extinction trial) X 2 (Condition) X 2 (Gender) Mixed ANOVA of ratings of sexual arousal, yielded a significant interaction for Stimulus X Phase

X Gender, $F(1, 94) = 5.69$, $p = .02$, $\eta_p^2 = .06$. Subsequent analysis of the preconditioning phase (Mean trial 1-4) and the first extinction trial for men and women separately, yielded significant interactions for Stimulus X Phase for men, $F(1, 36) = 6.73$, $p < .02$, $\eta_p^2 = .16$, and women, $F(1, 58) = 38.20$, $p < .01$, $\eta_p^2 = .40$, indicating conditioned responding. However, as can be seen in Figure 5, women displayed a stronger conditioned responding. Moreover, in line with the expectation, the analysis of the extinction phase yielded a significant Stimulus X Trial interaction, $F(4, 404) = 6.93$, $p < .01$, $\eta_p^2 = .07$, meaning a decrease of conditioned responding over trials. No Stimulus X Trial X Condition interaction was found, $p = .96$, but again a significant interaction for Stimulus X Trial X Gender, $F(4, 404) = 3.72$, $p < .01$, $\eta_p^2 = .04$. For subjective sexual arousal, both conditions did not differ in loss of differential responding, that is extinction. However, women showed a greater loss of differential ratings to CS+ and CS- during the extinction phase than men, as can be seen in Figure 5. Analysis of the first and last extinction trial yielded a significant interaction effect for Stimulus X Trial, $F(1, 97) = 21.0$, $p < .01$, $\eta_p^2 = .18$, indicating extinction of conditioned subjective sexual arousal. No significant interaction effect was found for Stimulus X Trial X Condition, $p = .93$, indicating no differences between the conditions in extinction of conditioned responding. Again an interaction effect was found for Stimulus X Trial X Gender, $F(1, 97) = 7.32$, $p < .01$, $\eta_p^2 = .07$. Separate analyses for men and women for the first and the last extinction trial were conducted. For men, this analysis yielded no significant interaction effect for Stimulus and Trial, $p = .27$, and Stimulus X Trial X Condition interaction, $p = .80$, meaning no extinction, with no differences between conditions. For women, this analysis yielded significance for Stimulus X Trial, $F(1, 60) = 37.22$, $p < .01$, $\eta_p^2 = .38$, meaning extinction of

conditioned differential responding. No differences between groups were observed in this loss of conditioned responding, Stimulus X Trial X Condition interaction, p= .84. Analysis of the first extinction trial yielded a significant main effect for Stimulus, $F(1, 97)$= 41.38, p< .01, η_p^2 = .30, and an interaction effect for Stimulus X Gender, $F(1, 97)$= 4.36, p= .04, η_p^2 = .04. Analysis of the last extinction trial also revealed a main effect for Stimulus, $F(1, 97)$= 9.67, p< .01, η_p^2 = .09, indicating there still was differential responding towards the CS+ and CS- on the last extinction trial, and men and women did no longer differ therein.

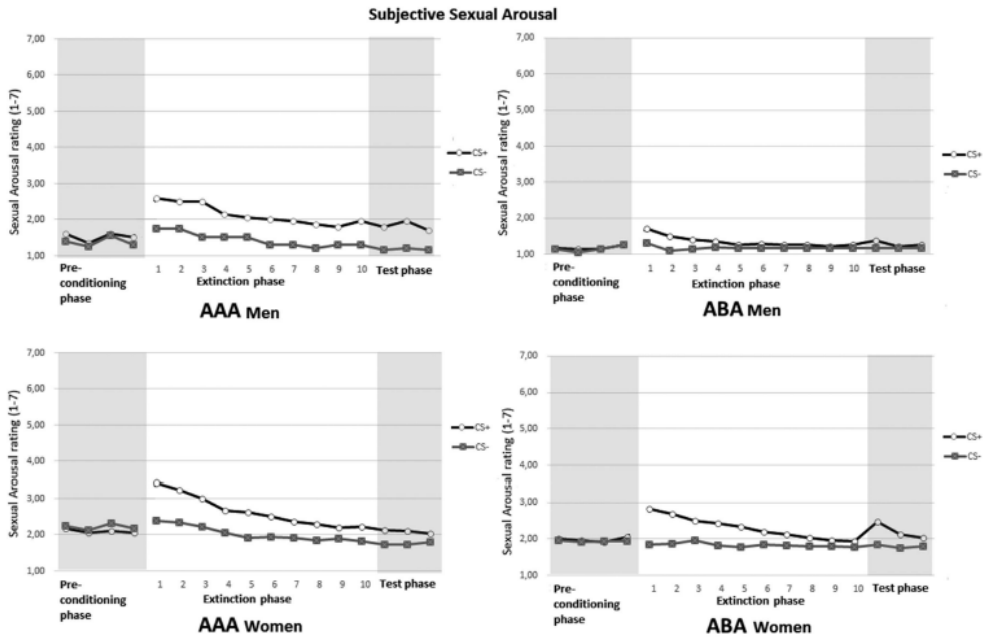

Figure 5. Ratings of subjective sexual arousal following the CS+ and CS- during the preconditioning phase, extinction phase and test phase for men (top) and women (bottom) in the two conditions AAA and ABA.

Test phase

US Expectancy The 2 (Stimulus) X 2 (Phase; last extinction trial and first test trial) X 2 (Condition) X 2 (Gender) Mixed ANOVA of ratings of US expectancy, yielded significance for Stimulus X Phase, $F(1, 94)= 10.01$, $p< .01$, $\eta_p^2 =.10$, and for the interaction of main interest, Stimulus X Phase X Condition, $F(1, 94)= 8.44$, $p< .01$, $\eta_p^2 =.08$. Subsequent analysis of the test phase yielded a significant interaction for Stimulus X Trial, $F(2, 153)= 9.11$, $p< .01$, $\eta_p^2 =.09$, and for Stimulus X Trial X Condition, $F(2, 153)= 8.31$, $p< .01$, $\eta_p^2 =.08$. As can be seen in Figure 3, men and women showed recovery of US expectancy towards the CS+ on the test trials, as result of context switch. Inspection of Figure 3 also suggests increased responding towards the CS- for men and women in the ABA condition. Additional analysis of the last extinction trial and first test trial for only CS- responses, yielded a significant interaction effect for men for Stimulus X Condition, $F(1, 94)= 12.05$, $p< .01$, $\eta_p^2 =.11$, indicating increased US expectancy would also follow the CS- as a result of context switch.

Affective Value The analysis of the last extinction trial and first test trial, yielded significant interactions for Stimulus X Condition, $F(1, 95)= 5.32$, $p= .02$, $\eta_p^2 =.05$, and most crucial to our hypothesis for Stimulus X Phase X Condition, $F(1, 95)= 5.76$, $p= .02$, $\eta_p^2 =.06$. Moreover, significant interactions were also found for Stimulus X Condition X Gender, $F(1, 95)= 4.21$, $p= .04$, $\eta_p^2 =.04$, and Stimulus X Phase X Condition X Gender, $F(1, 95)= 8.20$, $p< .01$, $\eta_p^2 =.08$. Since men did not show conditioned responding after the acquisition phase on affective value ratings, further results for men were not reported. Separate analyses for women, revealed a significant interaction effect, $F(1, 59)= 13.82$,

p< .01, η_p^2=.19. Inspection of Figure 4 suggests also increased responding towards the CS- on the first test trial for women in the ABA condition. Additional analysis of the last extinction trial and the first test trial for only affective value ratings towards CS- yielded a trend, F(1, 59)= 3.01, p< .09. Meaning affective value towards the CS- also increased as a result of context switch after extinction. Analysis of the test phase, yielded trends for Stimulus X Condition, p< .08, and for Stimulus X Trial X Condition, p= .07. Furthermore, the analysis yielded a trend for Stimulus X Condition X Gender, p<.06. The interaction effect of Stimulus X Condition indicates that the conditions differed in differential responding to the CS+ and CS-. The ABA condition showed recovery of conditioned responding and rated the CS+ as more positive as compared to the CS-. The significant interaction effect of Trial X Condition X Gender, indicates that there was a difference in responding between the two conditions between men and women, with only women showing recovery of conditioned responding, as can be seen in Figure 4. Additional analyses of the renewal phase for women, yielded significant interactions for Stimulus X Trial X Condition, F(2, 101)= 3.41, p= .04, η_p^2=.06.

Subjective Sexual Arousal Analysis of the last extinction trial and first test trial, yielded significance for Stimulus X Condition, F(1, 94)= 8.21, p< .01, η_p^2=.08, and most important, for Stimulus X Phase X Condition, F(1, 94)= 5.17, p= .03, η_p^2=.05. Also a significant interaction effect for Stimulus X Condition X Gender was seen, F(1, 94)= 5.41, p= .02, η_p^2=.05. Separate analyses for men and women, revealed no interaction effect for Stimulus X Phase X Condition in men, p= .54, whereas this analysis yielded a significant effect in women, F(1, 58)= 7.35 p< .01, η_p^2=.11, meaning increased conditioned responding after

context switch was observed only in women. Subsequent analysis of the test phase revealed significant interaction effects for Stimulus X Trial, $F(2, 175) = 7.64$, $p < .01$, $\eta_p^2 = .08$, and for Stimulus X Condition X Gender, $F(1, 93) = 4.63$, $p = .03$, $\eta_p^2 = .05$. Inspection of Figure 5 suggests these effects may be explained by larger responses by women to CS+ on the first test trial for the ABA condition, as compared to men. For men, additional analysis of the test phase, yielded no significant interaction for Stimulus X Trial, $p = .25$, whereas for women this interaction was significant, $F(2, 101) = 9.39$, $p < .01$, $\eta_p^2 = .14$. For men only a main effect for Stimulus was found, $F(1, 35) = 4.69$, $p < .04$, $\eta_p^2 = .12$.

6.3.3. Approach Avoidance Tendencies

t-tests were used to test if bias scores deviated significantly from zero within each condition, see Table 2. Differences in AAT bias scores were analyzed with mixed ANOVA with Gender and Condition as between-subject factor and Image as within-subject factor (CS+, CS-, CS-alike and neutral objects). Contrary to the expectations, no interaction effect was found for Image X Condition, $p = .28$. Participants from the two conditions did not differ in approach and avoidance tendencies across all stimuli. However, a main effect for Condition was found, $F(1, 95) = 5.17$, $p < .03$, $\eta_p^2 = .05$, reflecting more approach biases towards stimuli for participants in the ABA condition. Contrary to the expectations, there was no main effect for Image, $p = .62$, but a there was a trend for Image X Gender, $F(3, 258) = 2.39$, $p < .08$, meaning men and women differed in their bias scores. Further testing revealed that men and women differed in CS+ bias score, $t(97) = -2.20$, $p = .03$. Women were faster in approaching the CS+ as compared to men. They were however also faster in

approaching the CS- although this did not reach conventional level of significance, $t(97) = -1.66$, $p < .10$.

		Bias Score	M	SD	*p*
Men	**AAA**	**CS+**	17.8	44.8	*.10*
		CS-	15.6	60.3	*.27*
		CS alike	26.2	34.6	*< .10*
		Neutral	6.3	39.9	*.50*
	ABA	**CS+**	21.0	48.1	*.07*
		CS-	21.4	46.3	*.05*
		CS alike	23.9	47.6	*.04*
		Neutral	44.3	60.1	*< .01*
Women	**AAA**	**CS+**	30.2	53.7	*< .01*
		CS-	26.6	55.4	*< .02*
		CS alike	8.6	56.4	*.40*
		Neutral	10.4	48.4	*.26*
	ABA	**CS+**	54.7	56.8	*< .01*
		CS-	47.2	57.7	*< .01*
		CS alike	37.4	52.4	*< .01*
		Neutral	37.6	61.1	*< .01*

Table 2. One sample t-test results for Mean Approach Avoidance Task (AAT) bias score for CS+, CS-, CS-alike and neutral images in men and women in the AAA and ABA condition. Note: A positive score indicates faster reaction times on approach (pull) trials compared to avoid (push trials).

6.3.4. Correlations between Conditioned Responses

To investigate relationships between conditioned responses additional correlational analyses were conducted. We expected that the strength of the conditioned genital response would be positively related to the amount of change in subjective affect and subjective arousal and US expectancy. In addition, it was expected that the strength of the conditioned genital response would be positively related to the CS+ bias score. To investigate these relationships, for genital responses on SIR and TIR and ratings of affect, and

subjective sexual arousal and US expectancy, the difference between the response to the CS+ and the CS- during the first trial in the extinction phase was calculated by subtracting the response to the CS- from the response to the CS+. Pearson product-moment correlations between genital difference score during the first extinction trial, affect difference score, subjective sexual arousal difference score, US expectancy ratings difference scores, were calculated.

Table 3 shows that there were no significant correlations between the strength of the conditioned genital response and conditioned subjective and behavioural measures in men. However, in women, the strength of the conditioned genital response was correlated to the amount of change in subjective arousal and US expectancy. In addition, the strength of the conditioned genital response was also correlated to the magnitude of the CS+ bias score. Interestingly, the CS- bias score did not show such correlations.

		Affective Value	Subjective Sexual Arousal	US Expectancy	Conditioned Genital Response SIR	Conditioned Genital Response TIR	Bias Score CS+	Bias Score CS-
Men	**Affective Value**		.50**	.22	-.11	-.16	.17	.23
	Subjective Sexual Arousal	.50**		.29	-.15	-.14	-.21	-.08
	US Expectancy	.22	.29		.15	.10	-.13	-.34*
	Conditioned Genital Response SIR	-.11	-.15	.15		.91**	-.14	-.12
	Conditioned Genital Response TIR	-.16	-.14	.10	.91**		-.14	-.11
	Bias Score CS+	.17	-.21	-.13	-.14	-.14		.56**
Women	**Affective Value**		.13	-.34**	.15	.11	-.04	-.05
	Subjective Sexual Arousal	.13		.42**	.28*	.14	-.18	-.17
	US Expectancy	-.34**	.42**		.25	.27*	.07	.02
	Conditioned Genital Response SIR	.15	.28*	.25		.70**	.27*	.21
	Conditioned Genital Response TIR	.11	.14	.27*	.70**		.29*	.13
	Bias Score CS+	-.04	-.18	.07	.27*	.29*		.61**

Table 3. Correlations between conditioned genital response, conditioned affective change, conditioned subjective sexual arousal, conditioned US expectancy and conditioned approach and avoidance tendencies towards the CSs for men and women. ** $p > .01$; * $p > .05$

6.4. Discussion

The present study contributes to the growing literature on learning mechanisms in sexual behaviors, and provides support of the central feature of Bouton's theory of context dependency of extinction and renewal of conditioned responding in humans. We found evidence for this theory that an extinction procedure indeed does not erase conditioned sexual associations in humans but instead involves new learning that is context dependent. Changing context after an extinction procedure resulted in a significant increase of subjective affect and subjective sexual arousal in women and increased US expectancy ratings to CS+ as compared to CS- in both men and women (ABA condition), whereas no such recovery was observed in the absence of a context change (AAA condition). These results are important, because so far, context dependency of extinction in the sexual domain has not been studied in human studies.

However, it is crucial to mention that not all hypotheses were confirmed. First, no evidence for renewal was found for genital measures in men and women. For men, this can be explained by the fact that genital conditioning effects were not obtained. We will set out possible causes thereof hereafter. To be able to test for renewal, acquisition of conditioned responding has to be ascertained during the acquisition phase. Similarly, this also explains the finding that men did not show renewal of conditioned subjective affect during the test phase. However, although women showed conditioned genital responding, no renewal of such responding could be observed. For women the absence of renewed genital conditioned responding can be explained by the fact that this is complicated when extinction of such responding is not completely ascertained during the extinction phase. Since women showed no complete extinction of differential genital responding it is not entirely surprising no renewal was observed. In a similar manner, as men did not demonstrate extinction of conditioned subjective sexual arousal, renewal of conditioned responding was made harder to detect during the test phase.

As mentioned before, men did not show conditioned subjective affect. It can be speculated that the difference in US-evaluation between men and women can account for this. It appears that the vibrostimulation was a more effective sexual stimulus for women than for men, resulting in the absence of conditioned male genital response. Rowland and Slob (1992) demonstrated that penile vibrotactile stimulation significantly augments erectile response in the presence of an erotic videotape in healthy, sexually functional men. However, they found vibrotactile stimulation alone to produce the lowest level of genital and subjective sexual arousal compared to erotic film. In the present study, men declared to have liked the vibrostimulation as much as women did. Making it not entirely plausible for the vibrostimulation to have less sexual arousing properties for men, also reflected by clear conditioning effects on subjective measures of sexual arousal. Nevertheless, future studies on male sexual learning may consider vibrotactile stimulation combined with erotic film clips as US. In addition, it is suggested women have more erotic 'plasticity' (Baumeister, 2000), and men are more responsive to explicit erotic visual stimuli (Hamann, Herman, Nolan & Wallen, 2004). Results from the present study and another study from our lab (Brom et al., in preparation) support this notion. Using the same paradigm, but with sexually relevant CSs as the only difference, robust conditioned genital and subjective sexual arousal and affect was observed also in men, while making use of the same US. Therefore it seems that combination of a non-visual sexual US and neutral CSs is not sufficient to elicit conditioned genital responding in men. With respect to genital arousal, the present study contributes to the accumulating evidence (Both et al., 2008; Both, Brauer & Laan, 2011) that women can be sexually conditioned to initially neutral stimuli, whereas our results do not support such a straightforward mechanism in men, at least, when making use of a tactile US. However, making use of sexually explicit visual stimuli as US, conditioned responses towards an initial neutral CS (a penny jar) were observed by Plaud and Martini (1999). It could be that once

sexual preferences are established (Sisk & Foster, 2004), men are less susceptible to sexual learning to cues that differ too much from their developed preference (Chivers, Seto, Lalumière, Laan, & Grimbos, 2010; Coria-Avila, 2012; Pfaus, Kippin & Centeno, 2001). However, future research in men and women, making use of both neutral and sexual relevant CSs and visual and vibrotactile USs should be done to be conclusive about this.

Although the finding that men did not show conditioned genital response is in line with earlier sexual conditioning studies (Hoffmann, Janssen & Turner, 2004), these findings oppose the existing idea that men are more receptive to sexual conditioning than women (Brom, Both, Laan, Everaerd & Spinhoven, 2014; Pfaus, Kippin & Centeno, 2001). More studies on sexual learning in both sexes are needed before we can draw any firm conclusions about gender differences in sexual conditionability. The observed differences between men and women may not reflect pure gender differences in sexual conditionability, but may also be explained by differences in sample size and US effectiveness. In addition, we also should mention that sexually conditioned responses have generally been found to be small, especially with a neutral CS (Hoffmann, Janssen & Turner, 2004; O'Donohue & Plaud, 1994). For example, in their sexual conditioning experiment, Klucken et al. (2009) did also not find CRs (n=40), but making use of an increased number of participants (n=100) Klucken et al. (2013) did. Therefore, an explanation for the missing results could be decreased power.

This study is the first investigating whether initially neutral cues will elicit approach tendencies through their mere pairing with a sexually rewarding outcome. Contemporary emotion theories propose that sexual arousal, like any emotion, is a composite of subjective experience, physiological activity, and action disposition (Everaerd, 1988; Janssen, Everaerd, Spiering & Janssen, 2000; Mauss & Robinson, 2009). Some theorists state emotions are primarily action tendencies that are reflected in physiological activity and subjective

response (Frijda, 2010; Lang, 1985). In such a framework, the fact that a CS elicits sexual arousal response after pairing with a sexually rewarding US implies that the CS also elicits an approach tendency: the approach tendency installed through Pavlovian reward learning is translated into overt action. Although women in the AAA condition had an approach bias towards the CS+ and CS-, and ABA condition towards all stimuli, in the present study, men and women differed in implicit approach tendencies towards the stimulus that was paired with vibrostimulation, with women significantly faster approaching the CS+ than men. In women the CS+ elicited a more robust sexual arousal response as compared to men. This conditioned female sexual response translated into subjective experience, physiological measures and in action disposition. Given the finding that a less robust conditioned male sexual response was observed, strong approach tendencies could not be expected.

Contrary to expectations, but in line with results from another conditioning study from our lab (Brom et al. in preparation), men showed a smaller penile circumference in response to the CS+ compared to the CS- during the acquisition on the timeframes during vibrostimulation and also on timeframes when vibrostimulation no longer was applied. This finding does not lend itself to unambiguous interpretation. However, former research on automatic processing of sexual stimuli also found male genital responses to be opposite to the predictions: genital responses towards sexually primed targets were lower than responses to neutrally primed targets (Janssen, Everaerd, Spiering, Janssen, 2000). Those results were explained by physiological processes of penile erection. During the initial phases of erectile response, the penis undergoes an increase in length, and this is associated with a simultaneous decrease in circumference. Therefore, the physiology of penile erection may also account for the results found in the present study.

Quite puzzling is the observation of significant renewal effects for the CS- were observed on different measures. Vervliet, Baeyens, Van den Bergh

and Hermans (2013) noted that this increase in responding is quite common in studies on human spontaneous recovery and reinstatement. They suggested that this increased responding to the CS- can be explained by the CS- no longer being a neutral control stimulus in the test phase. It is possible that in the acquisition phase the CS- acquires inhibitory associations with the US. As a consequence of context change, this inhibition may be disrupted. According to Vervliet and colleagues the CS- may therefore not be the best control stimulus, as it may share the basic process of extinction: inhibition.

A limitation of the present study is the absence of a between subjects (unpaired) control group. Without such a control group it is difficult to determine whether and what learning has occurred, especially for men. At present it is unclear if the increased genital arousal towards the CS+ and CS- was due to conditioning or to pseudo conditioning. The possibility of sensitization of sexual arousal would translate into increased genital responses across trails, and not in differential responding towards the CS+ and CS- per se (Domjan, 2010; Hoffmann, Goodrich, Wilson & Janssen, 2014). Therefore, making use of such a control group in future research is desirable.

In line with earlier research on conditioning of appetitive responses (van Gucht, Vansteenwegen, Beckers & Van den Bergh, 2008), we demonstrated that not all behavioral and emotional changes produced by classical conditioning are organized in the same fashion. One interesting possibility is that US expectancy and subjective ratings of the CSs are not as much influenced by nonspecific sensitization effects of the US. As expected, results from the present study demonstrated that participants can learn to expect to receive a sexual reward when presented the CS+ and not to receive sexual reward when presented the CS-. Our data suggest that conditioned subjective affect and arousal, and conditioned approach tendencies and genital arousal differ from conditioned US expectancies. This divergence may reflect a more fundamental difference, which raises the question of whether there is

evidence for similar discrepancies between such measures in other appetitive paradigms (e.g. nicotine addiction). However, our data did demonstrate that in women conditioned US expectancy is correlated with conditioned affective value, conditioned subjective sexual arousal and conditioned genital arousal. In men, conditioned US expectancy was slightly correlated with conditioned subjective sexual arousal. Interestingly, in men, conditioned subjective sexual arousal is highly correlated with conditioned affective value, whereas in women it is not. This suggests that different response systems do not always behave in synchrony with each other in a sexual conditioning procedure: US expectancy, subjective sexual arousal and subjective affect may go hand in hand during this process of conditioning in men, whereas in women subjective sexual arousal does not seem to increase affective value, or vice versa. Further research should illuminate if this pattern is specific for sexual paradigms or if those behavioral and emotional changes produced by classical conditioning can be found in other appetitive conditioning procedures (e.g. substance addiction).

The present results may have implications for the treatment of sexual disorders with a learned component, like hypo- and hypersexuality. Extrapolating to clinical practice, the renewal of conditioned sexual responding may be observed in the relapse patients experience when leaving treatment context. Supported by results from the present study, it can be concluded that in the treatment of sexual disorders with a learned component it is important to reduce relapse after exposure treatment by generalization of extinction to other contexts and with multiple sexual stimuli. With respect to hypersexuality or paraphilia, this could mean applying treatment techniques in the context (e.g. a red-light district) in which the problematic behavior is experienced.

However, because it is evidently impossible to cover all sorts of situations or stimuli in therapy sessions, there will always be a certain risk for patients to relapse when confronted with a particular object, situation or mental state. Therefore, it may be a highly promising perspective to focus on processes

that modulate hippocampus-dependent contextual processing during extinction procedures. The glutamatergic N-methyl-D-aspartate (NMDA) receptor is considered essential for long-term potentiation, a process that underlies learning and extinction (Reichelt & Lee, 2013). D-cycloserine (DCS), a partial NMDA receptor agonist, has been shown to facilitate extinction of learned fear in rats (Ledgerwood, Richardson & Cranney, 2003; Walker, Ressler Lu & Davis, 2002), and in humans to facilitate extinction of fear and addictive behavior (Myers, Carlezon & Davis, 2011). The promising results from the studies on pharmacological agents in aversive extinction memory need to be replicated in appetitive conditioning paradigms, in order to know whether they are also applicable in extinction procedures of appetitive disorders.

In conclusion, this is the first observation of the renewal phenomenon of conditioned sexual responses and sexual reward expectancy in humans. The present research has demonstrated that genital and subjective sexual arousal seem to behave differently with regard to extinction and sensitivity to context changes. The results make clear that sexual arousal or the expectation of sexual reward can come under stimulus control by contextual cues associated with states of sexual reward. This makes clear that basic learning processes play a significant role in the development of human sexual behavior, and emphasizes the importance of future studies on sexual conditioning and related phenomena, and pharmacological influences thereof.

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Chapter 7

D-Cycloserine Reduces Context Specificity of Sexual Extinction Learning

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Abstract

D-cycloserine (DCS) enhances extinction processes in animals. Although classical conditioning is hypothesized to play a pivotal role in the aetiology of appetitive motivation problems, no research has been conducted on the effect of DCS on the reduction of context specificity of extinction in human appetitive learning, while facilitation hereof is relevant in the context of treatment of problematic reward-seeking behaviors. Female participants were presented with two conditioned stimuli (CSs) that either predicted (CS+) or did not predict (CS–) a potential sexual reward (unconditioned stimulus (US); genital vibrostimulation). Conditioning took place in context A and extinction in context B. Subjects received DCS (125mg) or placebo directly after the experiment on day 1 in a randomized, double-blind, between-subject fashion (Placebo n= 31; DCS n= 31). Subsequent testing for CS-evoked conditioned responses (CRs) in both the conditioning (A) and the extinction context (B) took place 24h later on day 2. Drug effects on consolidation were then assessed by comparing the recall of sexual extinction memories between the DCS and the placebo groups. Post learning administration of DCS facilitates sexual extinction memory consolidation and affects extinction's fundamental context specificity, evidenced by reduced conditioned genital and subjective sexual responses, relative to placebo, for presentations of the reward predicting cue 24h later outside the extinction context. DCS makes appetitive extinction memories context-independent and prevents the return of conditioned response. NMDA receptor glycine site agonists may be potential pharmacotherapies for the prevention of relapse of appetitive motivation disorders with a learned component.

7.1. Introduction

The glutamatergic N-methyl-D-aspartate (NMDA) receptor is essential in learning, memory, and experience-dependent forms of synaptic plasticity, such as long-term potentiation (LTP) (Reichelt & Lee, 2013). D-cycloserine (DCS) is a partial agonist at the NR1 NMDA receptor subunit and has been shown to enhance acquisition, consolidation, extinction and reconsolidation in several -especially aversive- associative learning paradigms in rodents and humans (Kalisch et al., 2009; Myers & Carlezon, 2012; Torregrossa et al., 2013). Although classical -or Pavlovian- conditioning is hypothesized to play a pivotal role in the aetiology of disorders such as addiction to substances, overeating (Robinson & Berridge, 1993; Jansen, 1998), and also in sexual motivation disorders, such as paraphilia and hypersexuality (Pfaus et al., 2001; Brom et al., 2014a), only little research has been conducted on the effect of DCS on human appetitive extinction learning, while facilitation of appetitive extinction learning is highly relevant in the context of treatment of for instance sexual motivation disorders, for which empirically validated treatments are lacking (Ter Kuile et al., 2009). Extinction is thought to be the core mechanism for widely used clinical interventions, such as cue exposure therapy, that reduce the impact of reward-associated cues in eliciting maladaptive learned responses, and involves repeated exposures to a cue in the absence of the event it once predicted (Delamater & Westbrook, 2014). However, extinction of conditioned responding is not the same as erasure, as conditioned responding is susceptible to renewal of conditioned responding as a result of context switch after extinction (Bouton, 2004; Brom et al., 2014b). Extrapolating the renewal phenomenon to clinical practice, someone who acquired craving for internet-sex at home (context A), and is successfully extinguished by cue exposure therapy in a therapeutic setting (context B), may experience strong craving upon changing context such as sitting behind the computer at home (context

A). Although generalization of extinction to other contexts and with multiple reward stimuli would be highly beneficial in reducing relapse, it is evidently impossible to cover all sorts of situations or stimuli in therapy sessions that patients might encounter in the future (Todd et al., 2014). Therefore, any pharmacological agent that that can render extinction context independent may provide an innovative method to reduce cue-induced relapse in the treatment of problematic reward-seeking behaviors.

In animals DCS has been shown to facilitate extinction of learned fear, to produce generalized extinction, and to reduce post-extinction reinstatement of fear (Reichelt & Lee, 2013), and in appetitive paradigms, administration of DCS facilitates the extinction consolidation of self-administration and conditioned place preference associated to different drugs (Myers & Carlezon, 2012). Although there are indications that DCS may primarily facilitate learning processes that underlie Pavlovian, rather than operant (i.e. instrumental action) extinction (Vurbic, Gold & Bouton, 2011), interestingly, DCS seems to enhance extinction of cocaine-associated cues in a novel context to reduce cue-induced reinstatement, meaning it reduces the context specificity of extinction (Torregrossa et al., 2010; 2013). In contrast to the animal literature, the DCS-augmentation effect for extinction learning and exposure therapy in humans is less consistent. In their meta-analysis, Ori and colleagues (Ori et al., 2015) found no difference between DCS and placebo in treatment outcome in anxiety and related disorders in children, adolescents and adults. The authors suggest this may partly due to low quality evidence from heterogeneous studies with small sample sizes and incomplete data for clinical response. However, there is some promising data that in humans DCS facilitates extinction of fear during cue– exposure therapy for a range of anxiety disorders (Fitzgerald et al., 2014), and limited studies have investigated DCS in treatment of substance-dependent subjects, with mixed results (Myers & Carlezon, 2012; Reichelt & Lee, 2013). However, the evidence for clinical efficacy of DCS in exposure therapy for

nicotine and cocaine addiction (Santa Ana et al., 2009; Price et al., 2013), combined with the results from animal studies (Torregrossa et al., 2010; 2013) provides a rationale for further investigation. To date, no investigation has determined whether DCS can reduce the context specificity of extinction of reward-associated cues in humans. This is especially relevant for the treatment of problematic reward-seeking behaviors, such as hypersexuality, for which empirically validated treatment is lacking (Kafka, 2007, 2010). In the present study, a differential sexual conditioning paradigm was applied, that has proven to be a fruitful paradigm for investigating human sexual reward learning (Both et al., 2011; Brom et al., 2014b). Contrary to stimuli, such as money, that gain reward value by learned associations with primary rewards, tactile sexual stimulation can be called a primary reward, because it does not require associative learning processes as it can reinforce behavior (Di Chiara, 1999; Schultz, 2006; Wise, 2002). Therefore, genital vibrostimulation served as US. The design consisted of sexual conditioning in context A and extinction in context B. It was hypothesized that administration of DCS after an extinction procedure will enhance extinction of conditioned sexual responses, reflected by a loss of conditioned genital and subjective sexual responding elicited by reward-conditioned cues in participants receiving DCS, even outside the extinction context, compared to participants in the placebo condition on a recall test 24h later.

7.2. Method

7.2.1. Participants

Sixty-two heterosexual women from the general population participated in the study, and gave written consent before participation. Subjects were pre-assessed by means of a telephonic interview to exclude those currently under any medication or treatment, those with past or present mental or neurological

illness, kidney impairment, those with a medical illness or use of medication that could interfere with sexual response or DCS, and allergy to antibiotics. Participants were tested individually by a trained female experimenter. The study was approved by the Ethical Committee of the Medical Centre. Participants were randomly assigned to one of the two treatment conditions Placebo or DCS, see Table 1.

7.2.2. Stimulus Materials (CSs)

Two identical pictures (Brom et al., 2015) served as CSs, and portrayed a male abdomen (wearing underwear), with the colour of the depicted underwear (Blue or Yellow) being the only difference. The CSs were shown for 9s. Assignment of the pictures as CS+ and CS- was counterbalanced across participants and conditions.

7.2.3. Genital Vibrostimulation (US)

The US was administered by means of a small hands-off vibrator (2 cm diameter) (see Both et al., 2011; Brom et al., 2014b). The vibrator was placed on the clitoris using a lycra panty that had an opening for the vaginal plethysmograph. The participants were instructed to place the vibrator in such a way it was most sexually stimulating. On day 1 the vibrostimulation was provided only during the acquisition phase, 8s following the start of the CS+ for 2s. A reinforcement ratio of 80% was chosen (8 out of 10 CS+ presentations are followed by genital vibrostimulation), to increase reward prediction uncertainty (Rescorla & Wagner, 1972; Schultz et al., 1997) in order to make conditioning somewhat more extinction resistant and increase the likelihood of recall of sexual reward memory on day 2. On day 2, recall of the sexual memory in context A was facilitated by additionally presenting unpaired US of 2s at the beginning of each context A block, thus again firmly associating context A with the US.

7.2.4. Context Manipulation

To investigate whether DCS can reduce context specificity of extinction of reward-associated cues in humans, conditioning and extinction occurred in 2 different contexts in order to create a context-dependent extinction memory. Contexts were manipulated by illuminating the experimental room in either a pink or a yellow light (Brom et al., 2014b). Lighting was supplied by a frame with six fluorescent tubes of 36 W (two pink and four yellow tubes). The experimenter controlled the lighting from an adjacent room. The colours of the lighting that served as Contexts A and B were randomly counterbalanced across participants.

7.2.5. Genital Arousal

Vaginal photoplethysmograph assessed vaginal pulse amplitude (VPA) (Laan et al., 1995). The photoplethysmograph is a menstrual tampon-sized device containing an orange-red light source and a photocell. The light source illuminates the capillary bed of the vaginal wall and the blood circulation within it. Depth of the probe and orientation of the light emitting diode were controlled by a device (a 6- X 2-cm plate) attached to the cable within 5 cm of the light sensor. The photoplethysmograph was disinfected at the medical centre by means of a plasma sterilization procedure between uses. Plasma sterilization is a highly effective method for the complete removal of all organic (and certain in-organic) material.

7.2.6. Subjective Ratings

Ratings of affective value, sexual arousal and US expectancy were collected during the preconditioning- and extinction phase on day 1 and during all context blocks on day 2. Participants were asked to rate after each CS presentation, the affective value of the CSs by answering the question "*What kind of feeling does this picture evoke in you*?" The question could be answered on a

seven-point Likert scale on a keyboard that varied from *very negative* to *very positive*. Then, subjective sexual arousal was rated by answering the question "*How sexually arousing is this picture to you*?" The question could be answered on a seven-point scale that varied from *not sexually arousing at all* to *very sexually arousing*. Then, participants were required to rate the expectancy of a vibration following the presentation of each CS on a seven-point scale by answering the question "*To what extent did you expect a vibration after this picture*"? The scale consisted of seven points labeled from '*certainly no vibration'* through '*certainly a vibration'*. The questions were presented at the monitor 1s following the end of picture presentation. The time the question was shown was paced by the participant's response; the time to respond was maximally 11s. When the participant answered the first question, the next question was presented after 15s.

7.2.7. Drugs

D-Cycloserine (DCS; King Pharmaceuticals, Leicester, UK) was orally administered as 1 capsule of 125mg. Optimal dosing for DCS has not been established in experimental human studies (Kalisch et al., 2009; Myers & Carlezon, 2012). Clinical studies suggest only moderate doses (50-125mg) DCS facilitate NMDA receptor dependent forms of synaptic plasticity as well as learning and memory (Rouaud & Billard, 2003). DCS plasma concentrations peak within 2h in sober subjects (Van Berckel et al., 1998). Therefore, subjects were asked not to eat 2h preceding the experiment, in order to facilitate DCS absorption and to assure high DCS plasma levels during the theoretical critical time window for NMDA-dependent memory consolidation of 1- to 2h post learning (Scavio et al., 1992; Van Berckel et al., 1998; Zhu et al., 2001). Subjects were asked to refrain from alcohol and other drugs on the evening before, and during the experimental days. Capsules with microcrystalline cellulose served as placebo.

7.2.8. Design

The design consisted of sexual conditioning in context A and extinction in context B, see Figure 1. The corresponding context was already present at the beginning of each block 8s before CS presentation started. In the acquisition phase in context A, the CS+ and CS- were presented 10 times each and 8 out of 10 CS+ presentations were followed by the US. The extinction phase in context B consisted of 10 unreinforced CSs presentations. There were two random orders for each phase; with the restriction of only two successive presentations of each CS. There was no interval between the preconditioning, acquisition, and extinction phases. During the whole procedure inter-trial intervals (ITIs) were 20, 25, or 30s. The order of the length of the ITI was random, with the restriction of only two successive lengths.

To ascertain retention of sexual extinction memories on day 2, conditioning and extinction was repeated in a further block. Subjects received either DCS or placebo directly after the experiment on day 1 in a randomized, double-blind, between-subject fashion (Placebo n= 31; DCS n= 31). Testing for CS-evoked conditioned responses (CRs) in both the conditioning (A) and the extinction context (B) took place 24h later on day 2. Each context (A and B) was presented 14 times each, in alternating order (ABAB…) and in each context 1 CS+ and 1 CS- was presented. At the beginning of context A, subjects received an unpaired US of 2s (i.e. not paired with the CS+ or CS-). Drug effects on consolidation were then assessed by comparing the recall of sexual extinction memories between the DCS and the placebo groups. Genital responses, assessed by vaginal photoplethysmography 13-16s following CS onset (Brom et al., 2014b) were acquired as a behavioral measure of physiological sexual arousal that may relate to sexual reward anticipation. Ratings of affective value, subjective sexual arousal and US expectancy were obtained after each CS-presentation in the preconditioning and extinction phases on day 1, and after each CS-presentation on day 2.

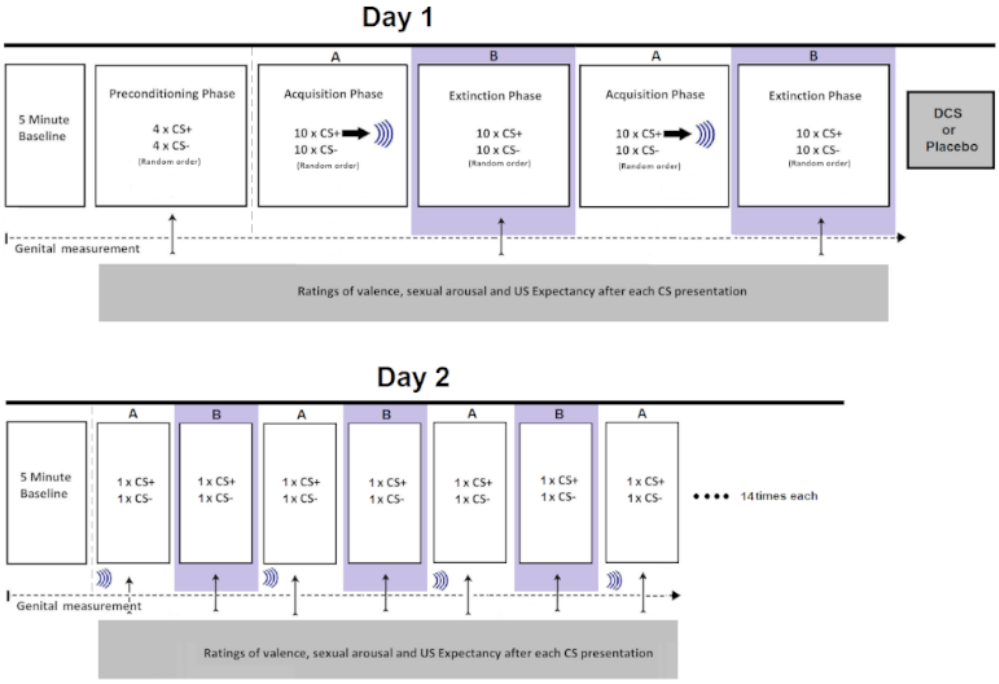

Figure 1. Schematic overview of the experimental procedure. **Day 1:** In the preconditioning phase, participants saw four (nonreinforced) presentations of each CS. In the acquisition phase in context A, the CS+ and CS- were presented 10 times each and 8 out of 10 CS+ presentations were followed by the US. The extinction phase in context B consisted of 10 unreinforced CSs presentations. To ascertain retention of sexual extinction memories on day 2, conditioning and extinction was repeated in a further block. Contexts were manipulated by illuminating the experimental room in either a pink or a yellow light. The last extinction phase was followed by administration of placebo or DCS. **Day 2:** CSs were presented in both contexts A and B to test for CS-evoked sexual extinction memory recall. Recall of the sexual memory in context A was facilitated by additionally presenting unpaired vibrostimulation of 2s at the beginning of each context A block, thus again firmly associating context A with the US. Waves denote genital vibrostimulation (US).

On day 1, 40 minutes after drug intake, participants filled in an adverse symptoms checklist, for physical symptoms like dizziness, nausea, and headache on a 4-point Likert scale (rated from 1 not present, 2 mild, 3 moderately severe, 4 extremely severe). On both days, after the experimental procedure, an exit interview questionnaire was administered. Participants were asked about the use

of the genital device, and their evaluation of the vibrotactile stimulus, and whether they had noticed the relationships between the CSs and US and contexts. Sixty minutes after drug intake, participants were allowed to leave the department.

7.2.9. Data Reduction, Scoring and Analysis

A software program (VSRRP98; University of Amsterdam) was used to reduce the genital data. After artefact removal, mean VPA level during the 2-minute resting baseline period was calculated. Genital responses to the CSs were scored in the latency window 13-16s following CS onset (Brom et al., 2014b; 2015). Change scores were calculated for each CS presentation by subtracting mean genital resting baseline from genital measurements following CS presentation. All phases were analysed separately. Acquisition of conditioning effects were tested with mixed factor univariate analysis of variance procedures (General Linear Model in SPSS) with Stimulus and Trial as within-subject factors, and Condition (DCS or Placebo) as between subjects factor. On day 1, early and late experimental extinction phases were analysed separately (Kalisch et al., 2009), and this was done by analysing the first and the last extinction trial of each phase. The Greenhouse–Geisser correction was applied to adjust for violation of the sphericity assumption in testing repeated measures effects. On Day 2, effects were tested with mixed factor univariate analysis of variance procedures (General Linear Model in SPSS) with Stimulus, Context and Trial as within-subject factors, and Condition (DCS or Placebo) as between subjects factor. All tests are two-tailed, and effect sizes are reported as proportion of partial variance (η_p^2). With a chosen *p*-value of .05, a power of 80% and an effect size of .5, a minimal number of 26 subjects was needed for within-subject effects (Cohen, 1988). Recent conditioning studies (Brom et al., 2014b; 2015) demonstrated that 30 subjects within each condition are sufficient to observe

between subjects-effects. In addition, studies on the effects of DCS on extinction (Kalisch et al., 2009; Santa Ana et al., 2009; Price et al., 2013) were able to detect between subjects-effects making use of 5-16 participants per condition. Inclusion of 62 women ensured a minimum of 30 women per condition after possible failure rate.

7.2.10. Efficiency of Blinding

Participants were asked 60 minutes after ingestion of the drugs on day 1, and before the experimental procedure on day 2 whether they thought they had received drug or placebo. Out of 62 subjects 6 (10%) answered they did not know. Thirteen (42%) participants from the DCS condition correctly guessed that they had received the drug, whereas 15 (48%) DCS participants incorrectly guessed that they had received placebo. Fourteen (45%) placebo subjects correctly guessed that they had received placebo, whereas 14 (45%) placebo subjects incorrectly guessed that they had received drug. This indicates that there was no relationship between the medication the participants had received and the percentage that correctly guessed what they had received (p=.79), suggesting that blinding was adequate. Most participants reported no side effects (n=42). Among the 20 participants (Placebo n=12; DCS n= 8) who reported side effects, the most commonly reported ones were lack of energy and sleepiness.

7.3. Results

Variable	Placebo (n= 31)		DCS (n= 31)		
	M	SD	M	SD	*p*
Age (years)	22.52	3.78	23.55	4.35	.32
Sexual Functioning (FSFI-score)	24.87	5.10	26.20	3.31	.28
Prior experience vibrostimulation	2.83	1.37	2.94	1.46	.78
Pleasantness US	3.33	0.71	3.42	0.72	.64
US perceived as sexually arousing	3.20	0.71	3.03	0.91	.43
Declared Sexual Arousal	2.68	0.79	2.45	0.81	.26
Strongest genital reaction	38.35	19.58	33.50	19.01	.33
Erotic fantasies	2.47	1.14	2.55	0.93	.76

Table 1. Descriptive subject variables. Notes: FSFI= *Female Sexual Function Index (Rosen et al., 2000; Ter Kuile et al., 2006)*. Questions from the Exit interview Day 1, Scales: Prior experience vibrostimulation: 1 (never) – 5 (very often); Pleasantness US: 1 (not pleasant at all) - 5 (very pleasant); US perceived as sexually arousing: 1 (not sexually arousing at all) – 5 (very sexually arousing); Declared Sexual Arousal (in response to US): 1 (no sexual arousal at all) – 5 (much sexual arousal); Strongest genital reaction in %; Erotic fantasies during the experiment: 1 (not at all) – 5 (very much).

7.3.1. Day 1: Sexual Conditioning and Extinction

Preconditioning Phase

Genital sexual arousal. Analyses were conducted to verify equal levels of VPA in response to the CS+ and CS- during the preconditioning phase. No difference in VPA following the CS+ or CS- was found, with no difference therein between the Placebo and DCS condition, $p>.20$.

Subjective measures. For affective value and subjective sexual arousal, no difference in responding following presentation of the CS+ and CS- was found

between the two conditions, all *p*s>.06. For US expectancy unexpectedly a main effect of Stimulus was found, $F(1, 56)= 4.16$, $p<.05$, $\eta_p^2 = .07$. US expectancy ratings were higher in response to presentation of the CS+ compared to CS-. No differences were seen between the two conditions, $p= .83$.

Acquisition Phases.

Genital sexual arousal. VPA in response to the vibrotactile stimulation during the acquisition phases was determined in order to verify whether the US elicited genital responses. In the first acquisition phase, a main effect of Stimulus was found, $F(1, 54)= 21.17$, $p<.01$, $\eta_p^2 =.28$, indicating that vibrostimulation resulted in a genital response, with no differences therein between the two conditions, $p=.37$. In the second acquisition phase, again an effect of Stimulus was found, $p< .01$, with no differences between conditions, $p>.08$.

Extinction Phases.

Genital sexual arousal. The mixed factors ANOVA with the genital CRs on the first extinction trial of the first extinction phase (B1) revealed conditioned responding, $F(1, 56)= 7.12$, $p=.01$, $\eta_p^2 =.11$, and on the last extinction trial extinction of CR was found, with no differences therein between conditions, all *p*s>.10. Analysis of the second extinction phase (B2) revealed no conditioning effects, and no differences between conditions, all *p*s>.30.

Subjective measures. Analyses revealed CRs on all subjective measures, all *p*s<.01, and subsequently extinction of CRs in both extinction phases, and no differences therein between conditions, all *p*s>.07. For depictions of genital and subjective CRs evoked by CS+ and CS– on day 1, see Figure 2 and 3.

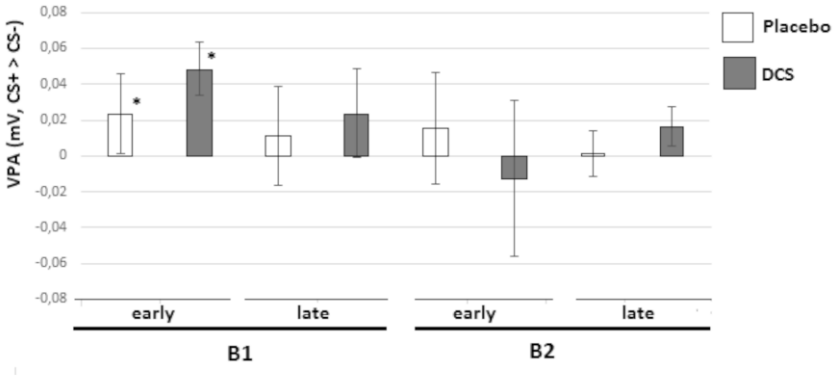

Figure 2. Vaginal Pulse Amplitude (VPA) Day 1. Mean Vaginal Pulse Amplitude (VPA) change scores from baseline (±S.E.M.) towards the CS + and CS- for the first 5 extinction trials (early), and for the last 5 extinction trials (late) in the first extinction (B1) phase and second extinction phase (B2). *****significant differential responding towards CS+ and CS-.

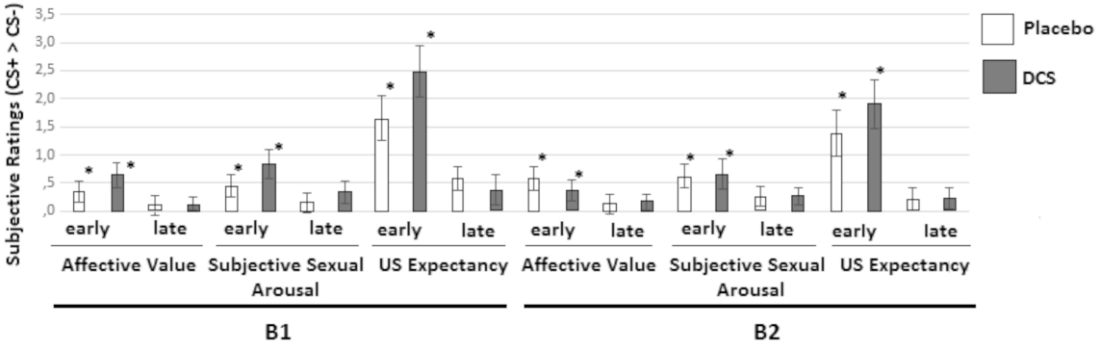

Figure 3. Subjective measures Day 1. Subjective measures CS+ > CS- scores (±S.E.M.) for the first (early) and last (late) extinction trial in the first extinction (B1) phase and second extinction phase (B2), for the placebo and DCS condition. *****significant differential responding towards CS+ and CS-.

7.3.2. Day 2: Recall of Sexual Extinction Memory

Genital sexual arousal. The mixed factor ANOVA with the genital CRs with Condition (placebo, DCS) as between-subject factor, and Stimulus (CS+ and CS-), Context (A, B) and Trial (14) as within-subject factors, revealed a main effect of Stimulus, $F(1, 53)= 5.33$, $p< .03$, $\eta_p^2 =.09$, indicating differential conditioned responding towards the CSs. Also a main effect of Context was found, $F(1, 53)=14.72$, $p< .01$, $\eta_p^2 =.22$. No Stimulus X Condition or Stimulus X Context X Condition interactions were found, ps$> .61$.

Planned Post-Hoc analysis (see Kalisch et al., 2009) of test trials in context A and B for both conditions separately revealed a main effect of Stimulus in the Placebo condition, $F(1, 52)= 4.86$, $p< .03$, $\eta_p^2 =.18$, whereas it did not in the DCS condition, $p= .35$. Main effects of Context were found, Placebo $F(1, 27)=10.89$, $p< .01$, $\eta_p^2 =.29$, DCS $F(1, 25)= 5.37$, $p< .03$, $\eta_p^2 =. 18$. Further analyses for both contexts separately, revealed only conditioned responding in the Placebo condition in the acquisition context A, $F(1, 27)= 5.65$, $p< .03$, $\eta_p^2 =.17$, DCS $p=. 25$. Both conditions did not show conditioned responding in the extinction context B, Placebo $p= .50$, DCS $p=. 70$. Figure 4 shows larger genital change scores (difference CS+, CS-) in context A for the Placebo condition, compared to the DCS condition.

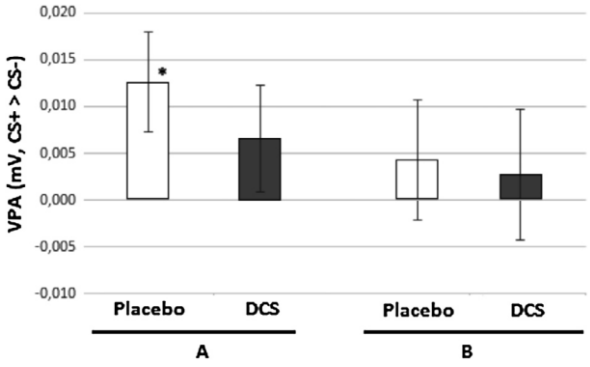

Figure 4. Mean VPA (Vaginal Pulse Amplitude) difference CS+ > CS- (±S.E.M.) on day 2 in the original acquisition context A, and in the extinction context B for the Placebo and DCS condition. *****Only participants in the Placebo condition demonstrated significant differential responding towards CS+ and CS- in context A.

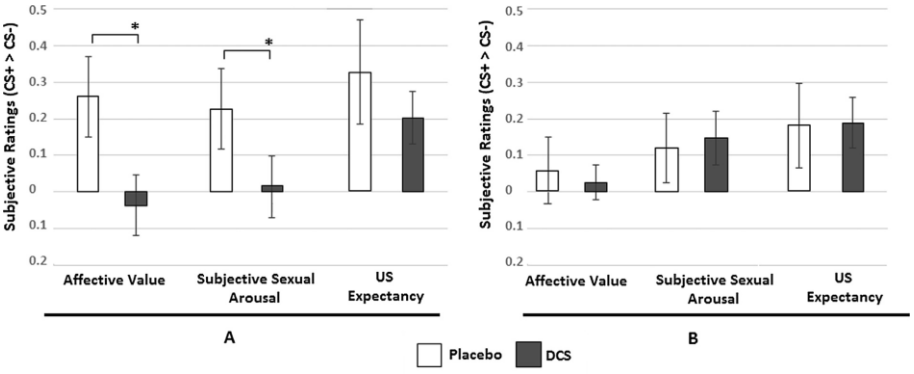

Figure 5. Effects of post learning DCS on subjective correlates (ratings of US Expectancy, Affective Value and Subjective Sexual Arousal; difference CS+ > CS-, and ±S.E.M) of recall of sexual extinction memory on day 2 in the original acquisition context A (left), and in the extinction context B (right). *CRs on Affective Value and Subjective Sexual Arousal showed a significant interaction between Stimulus (CS+, CS-) and Context (A, B) and Condition (Placebo, DCS).

Subjective measures. Both conditions did not differ in CRs on US expectancy, all *p*s>.12. Analyses for affective value ratings, revealed a significant Stimulus X Context X Condition interaction, $F(1, 48)= 4.43$, $p< .04$, $\eta_p^2 =.08$. As can be seen in Figure 5, the Placebo condition demonstrated larger CR scores (difference CS+, CS-) in context A, whereas participants in the DCS condition showed no conditioned responding. Analyses for both contexts separately, revealed a main effect of Stimulus in the Placebo condition in context A, $F(1, 24)= 5.59$, $p<. 03$, $\eta_p^2 =.19$, indicating differential responding towards the CS+ and CS-, whereas it did not in the DCS condition, $p= .67$. In context B no conditioned responding was found, Placebo $p=.52$, DCS $p=.56$. Analyses for both conditions separately, revealed no significant Stimulus X Context interaction effects, Placebo $p= .08$, DCS= .36. However, in both conditions main effects for Context were found, Placebo $F(1, 23)= 10.64$, $p< .01$, $\eta_p^2 = .32$, DCS $F(1, 25)= 12.37$, $p< .01$, $\eta_p^2 = .33$.

For subjective sexual arousal also a main effect of Stimulus was found, $F(1, 53)= 4.41$, $p= .40$, $\eta_p^2 = .08$, and a Stimulus X Context X Condition interaction, $F(1, 53)= 4.87$, $p= .03$, $\eta_p^2 =.08$. Figure 5 shows that only the Placebo condition had larger CR scores (difference CS+, CS-) in context A, whereas the DCS condition did not. In the Placebo condition, a significant interaction was found for Stimulus X Context, $F(1, 27)= 5.99$, $p= .02$, $\eta_p^2 =.18$, and a significant main effect of Context, $F(1, 27)= 12.50$, $p<.01$, $\eta_p^2 =.32$. Further testing revealed slight conditioned responding in context A in the Placebo condition, $F(1, 27)= 4.20$, $p= .05$, $\eta_p^2 =.14$, whereas it did not in the DCS condition, $p=.86$. Analysis of context B, revealed no conditioned responding in both conditions, Placebo $p=.22$, DCS, $p= .06$.

7.3.3. Sexual Reward-memory Recovery Index.

To test for recovery on Day 2 in a more stringent manner, a sexual reward-memory recovery index was calculated (Schiller et al., 2013): responses on the first trial in context A and in B on day 2 minus the last extinction trial on day 1 (B2) for each of the CS+ minus the CS−, see Figure 6. T-tests revealed there were no differences between the DCS and placebo condition in recovery index: US expectancy, context A, p= .38, context B p= .91; Affective Value, context A p= .19, context B p= .37; Subjective sexual arousal, context A p= .26, context B p= .73, although for genital arousal responses a trend was seen in context A; VPA context A, $t(48)$= 1.84, p= .07, context B, p= .53, suggesting a slight difference in recovery index between the DCS and Placebo condition in context A.

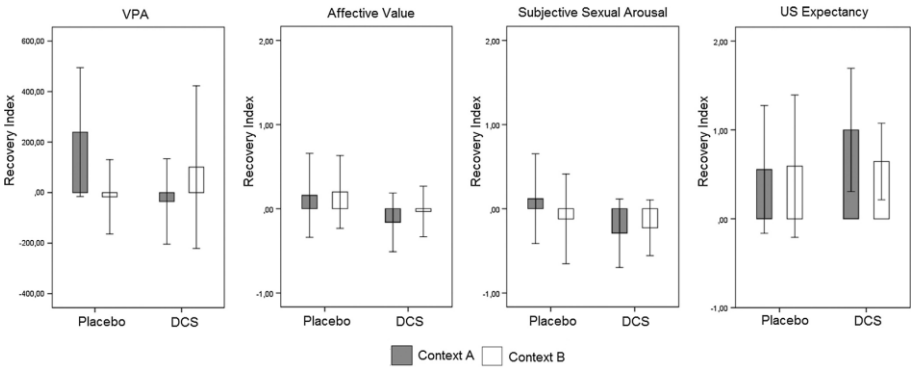

Figure 6. Recovery index: recovery in CR in the DCS and Placebo conditions (first trial day 2 minus last extinction trial day 1) for CS+ > CS− and ±S.E.M.

7.4. Discussion

This is the first study demonstrating that DCS affects extinction's fundamental context specificity in humans, at least in an (ABAB) appetitive sexual conditioning paradigm, since DCS enhanced extinction of conditioned responses also in the original acquisition context. This suggests that in humans, DCS makes extinction memories context-independent and prevents the return of conditioned response. However, results from the recovery index analyses suggest that these effects are small. Nevertheless, NMDA receptor glycine site agonists may be potential pharmacotherapies to reduce the motivational impact of reward-associated cues, and to prevent relapse in motivation disorders with a learned component.

From animal studies it is known that DCS facilitates fear extinction, but leaves animals vulnerable to renewal, suggesting that the effects of DCS were context-specific, at least in aversive paradigms (Woods & Bouton, 2006; Bouton et al., 2008). In line with results from appetitive conditioning studies in animals (Torregrossa et al., 2010; 2013), the present results suggest that DCS also affects extinction's fundamental context specificity in human appetitive conditioning paradigms. These results are highly interesting, especially when there is no *a priori* reason to believe that a drug that enhances extinction learning will change the nature of extinction learning qualitatively (Todd et al., 2014). One explanation can be that DCS enhances consolidation of the cue extinction memory, herewith making it stronger and more generalizable. However, results from aversive conditioning studies (Woods & Bouton, 2006; Bouton et al., 2008) are not in favour of this assumption. Another option can be that DCS interferes with context encoding in a way that the extinction memory is expressed independent of context. Indeed, research (Torregrossa et al., 2013) that examined the brain regions underlying animal appetitive Pavlovian cue extinction learning versus that which encodes the context associated with the cue extinction learning, demonstrated that NMDA receptor

antagonism in the nucleus accumbens (NAc) at the time of Pavlovian cue extinction training produced a subsequent increase in responding for conditioned reinforcement consistent with partial impairment in the learning/consolidation of the cue extinction memory. Interestingly, in this study a double dissociation was found that implicated the anterior cingulate cortex (ACC) in the encoding of contextual information during cue extinction, but not in encoding the cue extinction memory itself, whereas, the NAc is necessary for Pavlovian extinction learning. Inactivation of the ACC during cue extinction training prevented context appropriate expression of cue extinction learning when the animals were tested for renewal outside the extinction context. This corroborates results from the recent study on reward-motivated learning by Saez et al (2015). In this study monkeys performed an appetitive trace-conditioning task in which the sets of CS-US associations reversed many times for two CSs, creating two task sets, or contexts. Sometimes, a clear additional visual cue marked the context within a trial, but on the majority of trials, context was un-cued. Meaning, the monkeys had to use an internal representation of context to infer that the reinforcement contingencies of one CS had switched if they had first experienced the other CS-US pair after a reversal. In this study it was demonstrated that the neural representation of context emerges in the amygdala, orbitofrontal cortex, and ACC before a CS appeared, and is subsequently sustained during CS presentation, even when context is not cued by a sensory stimulus. Research suggests that ACC activations are important for discrimination learning (Martin-Soelch et al., 2007; Mechias et al., 2010), and traditionally it has been proposed that the amygdala and the ACC are densely interconnected (Ghashghaei et al., 2007). Saez et al (2015) suggest that the amygdala actively participates in maintenance of abstract relevant information, such as context. When reward memories are diminished through extinction (which relies on prefrontal-amygdala circuitry), the above suggests that the amygdala's and/or ACC's representations remain largely

intact, allowing the learned responses to recover (Schiller et al., 2013). Also Klucken and colleagues (2015) found the amygdala and ACC to be involved in the formation of reward-dependent memory. They investigated the association of Val158Met-polymorphism in the Catechol-O-Methyl-Transferase (COMT) and appetitive conditioning making use of a differential conditioning paradigm. This polymorphism is suggested to be associated with the alteration of neural processes of appetitive conditioning due to the central role of the dopaminergic system in reward processing. In this imaging study, they found a significant association between the COMT Val158Met-genotype and appetitive conditioning, since Val/Val-allele carriers showed increased hemodynamic responses in the amygdala compared with the Met/Met-allele group in the contrast CS+ vs CS-, and stronger hemodynamic responses in the ACC in Val/Val-allele carriers as compared to the Met/Met-allele group. The authors suggest that increased activity in amygdala and ACC combined with found increased hippocampal activity might reflect the interaction of these brain regions in forming reward-dependent long-term-memory of the CS+. Speculatively, DCS may impact the context dependency of appetitive extinction learning by acting on the amygdala and ACC. However, it is important to keep in mind that no imaging techniques were used in the present study. Therefore, this argumentation should be treated with caution until an independent replication is available. The mixed results from aversive paradigms on the effects of DCS on renewal of conditioned responding (Ressler et al., 2004; Woods & Bouton, 2006; Bouton et al., 2008) provide a rationale for further research to investigate if the context-a specific effect of DCS is limited to solely appetitive paradigms, herewith possibly indicating a fundamental difference in appetitive and aversive conditioned learning and extinction, and related neural circuits. Making use of imaging techniques, future studies should investigate which neural circuits are involved in appetitive and aversive extinction learning and in encoding of contextual information during extinction, and how these

circuits can be modulated to further improve the effectiveness of extinction based therapies.

It seems that extinction of conditioned US expectancy is not as much influenced by the effects of DCS as other measures of appetitive conditioned response. This divergence may reflect a more fundamental difference. Results from fear research suggest a dual-model theory of fear conditioning in humans that consists of two complementary defensive systems: a basic, lower-order, automatic process independent of conscious awareness, and a higher-order cognitive system associated with conscious awareness of danger and anticipation (Grillon, 2009: Kindt, Soeter & Vervliet, 2009; Haaker et al., 2013). Based on observations of the effects of DCS in animal and human studies, Grillon (2009) suggests that DCS influences extinction preferentially on lower- rather than higher-order learning. Since implicit associations and contingency awareness may be acquired independently (Bechara et al., 1995), and the latter implicates activity in higher order brain structures like the bilateral middle frontal gyrus and parahippocampal gyrus (Carter et al., 2006), it is possible that this involvement can explain the found insensitivity to the effects of DCS on this measure.

Although this study highlights the potential of DCS in reducing unwanted learned appetitive responses, there are some limitations of this study that must be considered before definitive inferences can be made. First, DCS has a plasma life of approximately 10-12h (Kalisch et al., 2009) while testing occurred after 24h. Research has shown that DCS at test may decrease conditioned (fear) responses (Ressler et al., 2004). Since only a moderate dose of 125mg was used in the current study, speculatively, the most likely explanation for the present results is the facilitatory effect of DCS on appetitive extinction memory consolidation, rather than on recall itself. Nevertheless, more research is needed, preferably testing for recall when participants are completely drug-free. Second, by using a combined conditioning and extinction

learning paradigm, it cannot be excluded that DCS interferes with both memory traces. However, since the aim of this experiment was to create context dependent acquisition and extinction memories, a possible influence of DCS on also the acquisition memory trace is not thought to hamper present results. Third, unpaired US presentations at the beginning of each former acquisition context A on day 2 likely induced reinstatement effects mixed with the contextual renewal effects (Kalisch et al., 2006; Haaker et al 2013). However, since sexual CRs have been found to be small (Hoffmann, Janssen & Turner, 2004; Brom et al., 2014b), in combination with the giving that any recall test in the absence of paired US-CSs is necessarily accompanied by ongoing extinction, a rationale was provided for introducing CR recovery over 14 context A blocks (see also Kalisch et al., 2009). A limitation of this study is therefore that we are unable to differentiate between renewal and reinstatement effects on recall of sexual memory. As a result only conclusions about the context-dependent recall of sexual extinction memory can be drawn. Future studies, testing for renewal effects in only one context (AAA-design) or in an additional context (ABC-design) are therefore warranted. Additionally, future studies should also investigate if similar results can be obtained without facilitating the recall of sexual memory on day 2 by presenting 1 unpaired US at the beginning of each context A block. Next, since the present study only investigated extinction of a sexual-reward conditioned cue, it is unclear if administration of DCS can also result in expression of extinction memory independent of context in other human appetitive learning paradigms, making use of artificial rewards, such as drugs, and other natural rewards, such as food. Therefore, future studies should examine whether it is possible to exploit these effects to facilitate extinction to prevent renewal of various reward seeking behaviours. Moreover, results from the recovery index analyses suggest that the effects of DCS on expression of sexual extinction memory are small, and for these stringent analyses, the current study seemed to be slightly underpowered.

Therefore, replication is needed, preferably making use of a larger sample size, and including men and women. The present study only included healthy sexually functioning women, and replication in men is necessary to investigate if DCS has the same effect on male sexual extinction memory. This is especially clinically relevant because disorders like hypersexuality and paraphilia are more prevalent in men than in women (Kafka, 2010), and this observation has led to the idea that men are more receptive to sexual conditioning than women, resulting in increased CR acquisition (Pfaus, Kippin & Centeno, 2001). Likewise, it would be interesting to investigate if DCS can also facilitate reward memory consolidation in the treatment of disorders characterized by low motivation or interest, such as depression, or in the sexual domain, such as low sexual arousal and interest disorder. Investigating the effect of administration of DCS after new learned appetitive sexual associations during cognitive behavioural treatment in disorders of low sexual arousal and interest may provide a promising perspective.

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SECTION 2

Chapter 8

The Influence of Emotion Down-Regulation on the Expectation of Sexual Reward

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Abstract

Emotion regulation research has shown successful altering of unwanted aversive emotional reactions. Cognitive strategies can also regulate expectations of reward arising from conditioned stimuli. However, less is known about the efficacy of such strategies with expectations elicited by conditioned appetitive sexual stimuli, and possible sex differences therein. In the present study it was examined whether a cognitive strategy (attentional deployment) could successfully down-regulate sexual arousal elicited by sexual reward-conditioned cues in men and women. A differential conditioning paradigm was applied, with genital vibrostimulation as unconditioned stimulus (US) and sexually relevant pictures as conditional stimuli (CSs). Evidence was found for emotion down-regulation to effect extinction of conditioned sexual responding in men. In women, the emotion down-regulatory strategy resulted in attenuated conditioned approach tendencies towards the CSs. The findings support that top-down modulation may indeed influence conditioned sexual responses. This knowledge may have implications for treating disturbances in sexual appetitive responses.

8.1. Introduction

Research in animals and humans support the notion that reward learning in the form of classical conditioning can contribute to the etiology of both normal and maladaptive sexual behaviors, like paraphilias, or deviant sexual preferences (Brom et al., 2014a; Pfaus, Kippin & Centeno, 2001). In classical conditioning, through the repeated association with the unconditional stimulus (US), a neutral stimulus (NS) can eventually elicit the same reaction as the US (Bindra, 1974; Pavlov, 1927). The NS is now called the conditioned stimulus (CS) and the reaction to the CS is called the conditioned response (CR). Several notable studies have demonstrated conditioned sexual arousal responses in humans (for a review see Brom et al., 2014a). Both from a learning theory and neuroscience perspective, disorders in sexual motivation, like hypersexuality, can potentially be characterized as disorders involving disturbed emotional learning and memory processes resulting in enhanced sexual response acquisition and maintenance.

The expectation of a potential sexual reward can elicit positive feelings and sexual arousal and therefore can aid in the learning about environmental cues that predict future sexual rewards. However, this reward expectation signal can also be maladaptive, potentially eliciting sexual urges that may be difficult to control, like in case of hypersexuality. Therefore, it is important to understand how to regulate or control the positive feelings associated with reward expectation. One promising method for examining this is the utilization of cognitive strategies. The term emotion regulation signifies any process that serves to initiate, inhibit or modulate (e.g. cognitively re-evaluate) emotional feelings or behavior (Aldao, 2013; Gross, 2002; Gross & Thomspon, 2007). Successful emotion-regulation may be dependent on top-down control from the prefrontal cortex over subcortical regions involved in reward and emotion. Failures in this deployment of top-down cognitive control mechanisms or

overactive bottom-up processes may contribute to several forms of psychopathology (Heatherton and Wagner, 2011; Ray and Zald, 2012), including sexual disorders with a learned component (Bancroft & Janssen, 2000; Both, Laan & Everaerd, 2011; Klucken et al., 2013; van Lankveld, van den Hout & Schouten, 2004; Salemink, van Lankveld, 2006). Cognitive strategies can successfully alter unwanted aversive emotional reactions. Emotional down-regulation strategies can influence emotions at the input phases (i.e. antecedent focused like cognitive reappraisal or attentional deployment) and at the output phase (i.e. response focused like suppression) (Gross, 1998; Webb, Miles & Sheeran, 2012). Gross and Thompson (2007) suggest that antecedent-focused strategies (e.g. attentional deployment) are more effective than response-focused strategies. As relatively few studies on negative emotions, and even less studies on positive emotions, have investigated the effects of the promising active distraction strategies (where the emphasis is on participants to bring to mind something unrelated to the emotion or emotional stimulus to serve as a distraction), especially on behavioral and physiological measures of emotion, this is an important avenue for future research (Webb, Miles & Sheeran, 2012). At present, there is growing evidence that cognitive strategies like attentional deployment can also regulate expectations of reward arising from conditioned stimuli (Delgado, Gillis & Phelps, 2008). However, less is known about the efficacy of such strategies with expectations elicited by conditioned appetitive sexual stimuli. To our knowledge, the present study is the first to investigate whether a cognitive down-regulatory strategy can efficiently regulate sexual arousal elicited by sexual reward-conditioned cues.

At present, it is unclear if men and women are equally prone to conditioning of sexual response and if sex differences do exist in the emotion regulation of positive emotions, like sexual arousal. Given the fact that paraphilia and hypersexuality are predominantly observed in men (Kafka 1994;

Kuzma & Black, 2008; Rosen, 2000) it is speculated that men are more receptive to increased CR acquisition (Domjan, 2005; Gutiérrez & Domjan, 1997; Klucken et al., 2009; Pfaus, Kippin & Centeno, 2001). Nevertheless, few studies have addressed sexual conditioning in both men and women (Brom et al., 2014a), and some results are contradictory to this general assertion (Brom et al, 2014b; Hoffmann, Janssen & Turner, 2004). Second, with respect to emotion regulation, the general assertion is that women use more emotion-focused strategies, while men are thought to use more efficient cognitive (rational) cognitive strategies (Whittle et al., 2011). However, most –if not all- of these results relate to the regulation of particularly negative emotions (Mak et al., 2009; McRae et al., 2008; Gross, 2007). Hence, the contradictory results of previous sexual conditioning studies and the lack of studies on sex differences in positive emotion regulation, point to the importance for further investigation of possible gender differences in sexual learning and cognitive regulation thereof.

In the present study, a differential conditioning paradigm was applied, with instructions adapted from Delgado, Gillis and Phelps (2008). It was predicted that participants in two conditions (the control condition *Attend* and the experimental *Down-Regulate* condition) would show conditioned genital and subjective sexual responding to the CS that was paired with the US (the CS+), which was expected to gradually decrease during extinction trials. When the *Attend* instruction preceded the CSs, the participant was instructed just to pay attention to the stimulus. In contrast, when the instruction *Regulate* appeared on screen, participants were instructed to conjure a soothing image from nature prompted by the colour of the stimulus. Instructions were presented in acquisition and extinction phases. It was predicted that an emotion down-regulation strategy would successfully decrease arousal elicited by the sexual reward-conditioned cue, in men and women, in both the acquisition and extinction phases. Since subjective ratings are susceptible to demand

characteristics, in addition a task was included to assess implicit approach and avoidance tendencies towards the CS (Cousijn, et al., 2011). We assumed participants should be faster when instructed to approach the CS+ and avoid from the CS- than when instructed to avoid the CS+ and approach the CS-, and an emotion down regulation strategy should decrease these responses elicited by reward-conditioned cues.

8.2. Method

8.2.1. Participants

Research participants were 40 men and 53 women. Participants were paid €30,- for their participation and were recruited using posted advertisements. The advertisement stated that the focus of the study would be on the relationship between erotic (genital) stimulation and sexual arousal. Inclusion criteria were: age between 18 and 45 years and a heterosexual orientation. Exclusion criteria were: sexual problems, a Diagnostic and Statistical Manual of Mental Disorders 4th Edition (DSM-IV) diagnosis of an affective or psychotic disorder or abusive drug use, pregnancy or breastfeeding, and a medical illness or use of medication that could interfere with sexual response. Written informed consent was obtained from all participants. The study was approved by the Ethical Committee of the Medical Center.

8.2.2. Design and Conditioning Procedure

One stimulus (the CS+) was followed by the genital vibrostimulation (US) during the acquisition phase, whereas the other stimulus (CS-) was never followed by genital vibrostimulation. Participants were randomly assigned to one of the two conditions: Down-Regulate or Attend, with restriction that conditions matched on sex as close as possible. For a schematic overview of the procedure see Figure 1. In the preconditioning phase, participants saw four

nonreinforced presentations of the CS+ and four presentations of the CS-, for 9 seconds each. Subsequently, in the acquisition phase the CS+ and CS- were presented 10 times each and the CS+ was always followed by the US. In the extinction phase the CS+ was no longer followed by the US. Prior to CS presentation, in the acquisition- and extinction phases participants were presented with a written cue (*attend* or *regulate*) on screen for 2 s that reminded participants to either Attend or Down-Regulate. All phases were presented without interruption. Genital response was measured continuously during resting baseline, preconditioning, acquisition, and extinction phases. There were two random CS orders for each phase (that was counterbalanced across participants); with the restriction of only two successive presentations of each CS. During the whole procedure inter-trial intervals (ITIs) were 20, 25, or 30 seconds. The order of the length of the ITI was random, with the restriction of only two successive lengths. Stimuli and cues were presented by using E-prime 2.0 Software (Psychology Software Tools, Inc).

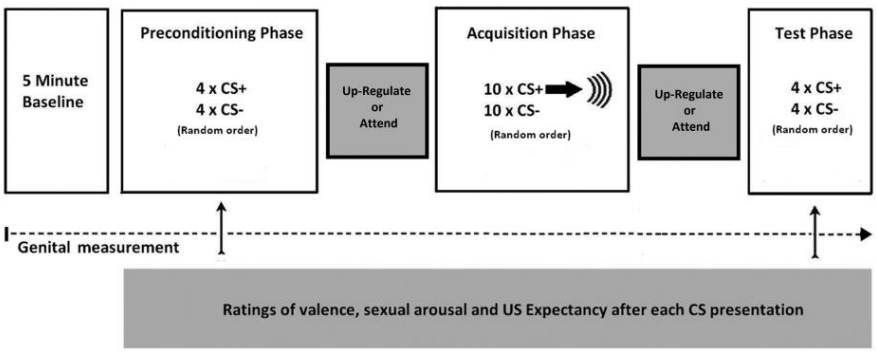

Figure 1. Schematic representation of the experimental procedure in both conditions. In the acquisition and extinction phase, before every CS presentation a written cue was presented: participants in the Down-Regulate condition received the instruction "Regulate" whereas participants in the control condition received the written cue "Attend" prior to each CS+. Assignment of the colour of the pictures (blue or yellow) as CS+ and CS− was counterbalanced across participants and conditions.

8.2.3. Materials, Apparatus, and Recording

Stimulus materials. Two identical pictures served as CSs, and portrayed an abdomen of an individual of the opposite sex (wearing underwear), with the colour of the underwear in the picture (Blue or Yellow) being the only difference. The CSs were shown in the middle of a computer monitor, approximately 1.5 m in front of the participant. The size of the presented pictures was 14 X 21 cm. Assignment of the pictures as CS+ and CS- was counterbalanced across participants and conditions.

Written instructions. In the *Attend* condition participants received the written cue *Attend* prior to each trial in the acquisition and extinction phases. They were instructed to '*just pay attention'* to the CSs when they were presented this cue. In contrast, in the Down-Regulate condition participants were only presented with the *Regulate* cue in the acquisition and extinction phases, and were instructed that when the cue *Regulate* appeared on the monitor, they should conjure a soothing image from nature prompted by the colour of the CS. For example, upon seeing the blue CS, participant could imagine the ocean or blue sky, while imagining a sunny beach or a field of flowers for the yellow CS. Participants were asked to generate the same image every time each colour CS was presented.

Genital vibrostimulation (US). Genital vibrostimulation was provided 8s following the start of the CS+ for 2s. For men, the US was administered by means of a ring-shaped vibrator. They were instructed to place the vibrator just below the coronal ridge (Janssen, 1994). For women, a small hands-off vibrator (2 cm diameter) was used (Laan & van Lunsen, 2002). The vibrator was placed on the clitoris using a lycra panties that had an opening for the vaginal

plethysmograph. All participants were instructed to position the vibrator as *most sexually stimulating*.

Male genital sexual arousal. An indium/gallium-in-rubber penile gauge assessed changes in penile circumference (Bancroft, Jones, & Pullan, 1966). The gauges were calibrated before each laboratory session using a set of calibrated rings (Janssen, Prause, & Geer, 2007). The penile gauge was positioned two-thirds of the way down the shaft of the penis toward the base. Changes in electrical output caused by expansion of the gauge were recorded by a continuous DC signal. The Indium-Gallium penile gauges were disinfected after each use, according to Sekusept plus disinfection procedure (MedCaT B.V.). Sekusept plus contains Glucoprotamine, which action spectrum covers bacteria including mycobacteria, fungi and viruses (e.g. Human Papillomavirus [HPV]) (MedCaT B.V.).

Women's genital arousal. Vaginal photoplethysmography assessed vaginal pulse amplitude (VPA) (Laan, Everaerd & Evers, 1995). Depth of the probe and orientation of the light emitting diode were controlled by a device (a 6- X 2-cm plate) attached to the cable. The vaginal photoplethysmograph was disinfected by means of a plasma sterilization procedure between uses. Plasma sterilization is a highly effective method for the complete removal of all organic (and certain in-organic) materials.

Subjective ratings. Ratings of affective value, sexual arousal and US expectancy were collected during the preconditioning- and extinction phases. Participants were first asked to rate, after each CS presentation, the affective value of the CSs by answering the question "*What kind of feeling does this picture evoke in you*?" The question could be answered on a seven-point Likert scale on a keyboard that varied from *very negative* to *very positive*. Then, subjective sexual

arousal was rated by answering the question "*How sexually arousing is this picture to you*?" The question could be answered on a seven-point scale that varied from *not sexually arousing at all* to *very sexually arousing*. Then, participants were required to rate the expectancy of a vibration following the presentation of each CS on a seven-point scale by answering the question "*To what extent did you expect a vibration after this picture*"? The scale consisted of seven points labeled from '*certainly no vibration'* through '*certainly a vibration'*. The questions were presented at the monitor 1 second following the end of picture presentation.

8.2.4. Other Measures

Approach avoidance task (AAT, see Cousijn et al., 2011; E-prime 2.0 Software, Psychology Software Tools, Inc)**.** Participants were presented with the CS+, CS-, and neutral pictures from the International Affective Picture System (IAPS; Lang, Bradley and Cuthbert, 2005). All images were rotated 3° left or right. Image content was irrelevant to the task: participants were instructed to pull or push the joystick in response to rotation direction. Pulling and pushing the joystick gradually increased and decreased image-size. The CS+, CS- and the neutral pictures were presented 80 times each, 40 times in push- and 40 times in pull-format, resulting in 240 test trials. The latency was recorded between picture onset and completion of a full push or pull response. Literature supports the AAT's validity in measuring approach/avoidance motivational processes (Wiers et al., 2011).

The international index of erectile function (IIEF). This is a validated 15-question questionnaire that examines 4 main domains of male sexual function: erectile function (6 questions, range 0-5), orgasmic function (2 questions, range 0-5), sexual desire (2 questions, range 0-5), and intercourse satisfaction (3

questions, range 0-5). Higher scores indicate better sexual function. Psychometric properties of the IIEF are good (Rosen et al., 1997).

The female sexual function index (FSFI). Women's sexual functioning was assessed by the FSFI (Rosen et al., 2000; Ter Kuile, Brauer & Laan, 2006), consisting of six subscales: desire (two items; range 1–5), arousal (four items; range 0–5), lubrication (four items; range 0–5), orgasm (three items; range 0–5), satisfaction (three items; range 0–5), and pain (three items; range 0–5). A higher score indicates better sexual functioning. The FSFI has good internal reliability and is able to differentiate between clinical samples and nondysfunctional controls.

Exit interview. Participants were asked, among others things, about their reactions to the experimental procedure, the use of the genital device, and their evaluation of the genital vibrostimulation. For instance, participants were asked to what extent they liked the vibrostimulation. This could be rated at a 5-point scale ranging from (1) not pleasant at all, to (5) very pleasant. Likewise, participants were asked how sexually aroused they became by the vibration. In addition, they were asked about any prior experience with vibrostimulation. Participants were also asked about the used cognitive strategies, and they were asked to rate how successful they were in concentrating and in the deployment of the cognitive strategy on a scale from 1 to 5 (i.e. 1 (trouble keeping concentrated) – 5 (well capable keeping concentrated); and 1 (not successful at all) – 5 (very successful).

8.2.5. Procedure

After participants completed the first session of the AAT, participants were instructed that the purpose of the experiment was to measure physiological responses to different pictures and to genital vibrostimulation. Before entering

the experimental conditioning session, participants were instructed about the vibrostimulation, the colours of the CSs, and the written cues that would appear on screen. Participants were made aware of the contingencies (e.g., only the colour blue or yellow predicted a potential genital vibrostimulation). Then *Attend* or *Regulate* instructions were explained. Participants were asked to verbalize what they were planning to think about when being presented with the written cues Attend and Regulate to assure that they were following the instructions they were given. In addition, participants were notified that regardless of the instruction, the CS+ always indicated the possibility of receiving genital vibrostimulation. Subsequently, the experimental conditioning experiment followed (see Both et al., 2008, 2011; Brom et al., 2014b for conditioning procedure), starting with the preconditioning phase, followed by the acquisition and extinction phases. In the acquisition and extinction phase participants were presented with the written cue Attend or Regulate prior to each CS. Directly after this experimental procedure, the second session of the AAT was completed. Then participants privately filled in questionnaires (e.g., FSFI, Rosen et al., 2000; IIEF, Rosen, 1997) and an exit interview questionnaire was administered.

8.2.6. Data Reduction, Scoring and Analysis

After artefact removal, mean penile circumference or mean VPA level during the 2-minute resting baseline period was calculated. Genital responses to the CSs were scored in three latency windows: during 4-8, 9-12 and 13-16 seconds following CS onset, respectively FIR (first interval response), SIR (second interval response) and TIR (third interval response) (see also Both et al., 2008; 2011) For FIR, SIR and TIR, change scores were calculated for each CS presentation by subtracting mean genital resting baseline from genital measures following CS presentation. Since direct gender comparison of genital responses

cannot be made because of the different measures used, genital data for men and women was analysed separately. For genital responses, effects were tested with mixed factor univariate analysis of variance procedures (General Linear Model in SPSS), with Stimulus and Trial as within-subject factors and Condition as between subjects factor. Analyses of subjective measurements and AAT scores were conducted for men and women combined, with Condition and Gender as between subjects factor (General Linear Model in SPSS). The Greenhouse–Geisser correction was applied to adjust for violation of the sphericity assumption in testing repeated measures effects. All phases were analysed separately. Preconditioning and acquisition phases were both analysed as a whole, whereas individual extinction trials were analysed separately, since sexual conditioning effects have generally been found to be small (Brom et al., 2014b; Hoffmann, Janssen & Turner, 2004), and the deployment of the emotion regulation strategy is expected to affect not only the magnitude of conditioned responding (trial 1 and 2 of the extinction phase) but also the extinction of conditioned responding (trial 3 and 4 of the extinction phase). Effect sizes are reported as proportion of partial variance (η_p^2) or as Cohen's *d* for paired comparisons (Cohen, 1988). Data from the AAT were corrected for outliers. Median RTs were used because they are less sensitive to outliers than means (see Cousijn et al., 2011). Bias scores (median push – pull) were computed for CS+, CS- and the neutral pictures. A positive bias score will be referred to further as an approach-bias and a negative bias score as an avoid-bias. AAT bias scores were analysed using standard analysis of variance (ANOVA).

8.3. Results

Participants were randomly assigned to one of the two conditions with the restriction that conditions were matched on sex as close as possible: Down-Regulate (N=46; Men, n = 20) and Attend (N= 47; Men, n = 20), see Table 1 *Subject characteristics*.

8.3.1. Genital Sexual Arousal

Preconditioning phase.

For all latency windows (FIR, SIR and TIR), no difference in circumference following presentation of the CS+ and CS- was found, all ps > .42. In addition, for women, on all time latencies, no difference in VPA following presentation of the CS+ and CS- was found, all ps > .20.

Table 1. Subject characteristics (p. 312)**.** Descriptive subject variables for men and women, and for each condition. Notes: IIEF= *International Index of Erectile Function* (Rosen et al.. 1997); FSFI= *Female Sexual Function Index* (Rosen et al., 2000; Ter Kuile, Brauer & Laan, 2006). Questions from exit interview. Scales: Prior experience vibrostimulation: 1 (never) – 5 (very often); Pleasantness US: 1 (not pleasant at all) - 5 (very pleasant); US perceived as sexually arousing: 1 (not sexually arousing at all) – 5 (very sexually arousing); Declared sexual arousal: 1 (not sexually aroused) – 5 (very sexually aroused); Instructions: Able to concentrate: 1 (trouble keeping concentrated) – 5 (well capable keeping concentrated); Instructions: successful deployment of cognitive strategies: 1 (not successful at all) – 5 (very successful); Examples of what participants thought of in the *Regulate* condition when presented with their CS+ are: seeing a blue sky with contrails, the sea, a yellow beach, or a yellow dessert. * p < .05.

Variable	Men						Women						Men & Women					
	Attend (n=20)		Down-Regulate (n=20)			Effect Size	Attend (n= 29)		Down-Regulate (n= 33)			Effect Size	Men (N= 40)		Women (N= 52)			Effect Size
	M	SD	M	SD	*p*	Cohen's *d*	M	SD	M	SD	*p*	Cohen's *d*	M	SD	M	SD	*p*	Cohen's *d*
Age (years)	25	6.1	22	2.6	.08	.66	28.8	8.2	27.7	8.1	.64	.14	23.6	4.8	28.2	8.0	< .01*	.70
Sexual Functioning (IIEF/FSFI score)	36.2	5.6	34.9	5.8	.69	.41	27.3	2.8	28.1	2.8	.32	.29						
Prior Experience Vibrostimulation	1.7	1.1	1.7	0.9	.96	.00	3.8	1.3	3.7	1.1	.87	.08	1.7	1.0	3.8	1.1	< .01*	2.0
Pleasantness US	3.2	1.4	3.3	0.9	.81	.09	3.5	0.7	3.2	0.6	.21	.47	3.2	1.2	3.4	0.8	.48	.03
US Perceived as Sexually Arousing	3.1	1.2	3.0	1.1	.89	.09	3.2	0.9	3.1	0.8	.90	.11	3.0	1.2	3.1	0.9	.59	.10
Declared Sexual Arousal	2.5	1.4	2.5	1.0	.95	.00	2.5	0.8	2.5	0.8	.86	.00	2.5	1.2	2.6	0.9	.66	.10
Instructions: Able to concentrate	4.3	0.5	3.93	0.6	0.9	.69	4.0	0.4	3.5	0.6	< .01*	.98	4.1	0.6	3.8	0.6	< .01*	.50
Instructions: Successful delployment of cognitive strategy	4.2	0.7	4.0	0.5	.36	.34	3.5	0.6	3.7	0.5	0.8	.37	.41	0.6	3.8	0.5	0.2	.56

Acquisition phase.

Men. Figure 2 summarizes penile circumference (SIR) to CS+ and CS- across trials for the conditions Attend and Down-Regulate. A main effect of Stimulus was found on FIR, $F(1, 38) = 8.29$, $p < .01$, $\eta_p^2 = .18$; and SIR, $F(1, 38) = 90.88$, $p < .01$, $\eta_p^2 = .71$, indicating the vibrostimulation resulted in a genital response. In line with Brom et al. (2014b) penile circumference was smaller in response to the CS+ and vibrostimulation than in response to the CS-. On TIR no main effect of Stimulus was found, $p = .23$. No differences in differential responding were observed between the conditions, FIR $p = .47$; SIR $p = .40$; TIR $p = .38$, and no main effect of Condition was found, FIR $p = .68$; SIR $p = .71$; TIR $p = .71$.

Women. Figure 3 summarizes VPA (SIR) to CS+ and CS- across trials for both conditions separately. In line with previous studies (Both et al., 2008; 2011), the 2 (Stimulus) X 10 (Trial) X 2 (Condition) mixed ANOVA of VPA revealed no significant main effect of Stimulus on FIR, $p = .07$, but did on SIR, $F(1, 51) = 18.77$, $p < .01$, $\eta_p^2 = .27$, and TIR, $F(1, 50) = 50.51$, $p < .01$, $\eta_p^2 = .50$. A Stimulus X Condition interaction was not found, FIR $p = .15$; SIR $p = .15$; TIR $p = .34$, nor of Stimulus X Trial X Condition, FIR $p = .25$; SIR $p = .59$; TIR $p = .38$.

Extinction phase.

Men. Analysis of the first extinction trial revealed a significant main effect of Stimulus on FIR $F(1, 38) = 4.19$, $p < .05$, $\eta_p^2 = .10$; and SIR, $F(1, 38) = 4.16$, $p < .05$, $\eta_p^2 = .10$, indicating conditioned responding. A Stimulus X Condition interaction was not found, FIR $p = .27$; SIR $p = .25$, TIR $p = .30$. Analysis of the entire extinction phase revealed overall smaller penile responses to CS+ than to

CS-, as reflected by the significant main effect of Stimulus on SIR, $F(1, 38) = 4.29$, $p < .05$, $\eta_p^2 = .10$, indicating conditioned responding. No interaction effects of Stimulus X Trial X Condition, and Stimulus X Condition were seen, all ps > .17. On FIR and TIR a significant interaction effect of Stimulus X Trial was found, FIR $F(2, 79) = 3.46$, $p < .04$, $\eta_p^2 = .08$; TIR $F(2, 80) = 3.07$, $p < .05$, $\eta_p^2 = .08$, indicating extinction. On the last extinction trial no significant main effect of Stimulus was found, FIR $p = .13$, SIR $p = .36$, TIR $p = .21$. Analysis of only responses towards the CS+ during the preconditioning trials and the extinction trials revealed no differences in conditioned responding between the Attend and Down-Regulate condition, as reflected by non-significant Trial X Condition interactions, all ps > .10.

Women. On the first extinction trial no significant main effect of Stimulus was found, FIR $p = .45$, SIR $p = .35$, TIR $p = .47$. No differences were seen between the conditions, Stimulus X Condition, FIR $p = .60$; SIR, $p = .88$; TIR $p = .98$. Analysis of the entire extinction phase, revealed no significant effect of Stimulus, FIR $p = .97$, SIR $p = .13$, TIR, $p = .71$. Analysis of the preconditioning phase (MEAN precon 1-4) and the first extinction trial demonstrated no significant main effect of Stimulus, with no differences between the two conditions, all ps > .31. On the last extinction trial, no significant main effect was found of Stimulus, FIR $p = .74$; SIR $p = .61$; TIR $p = .54$, and no differences therein between conditions, all ps > .18.

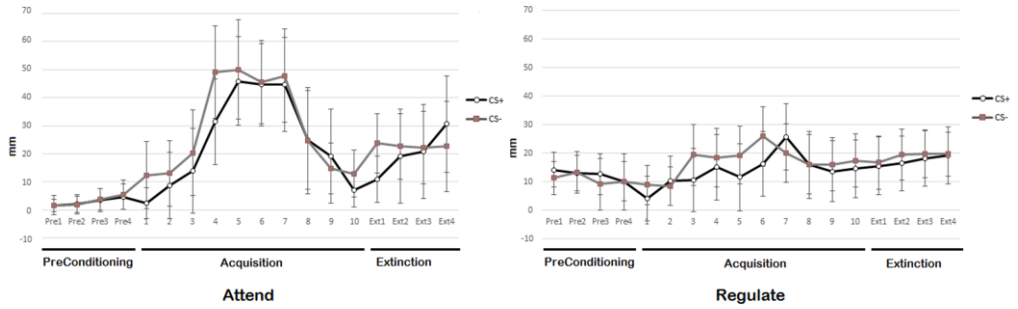

Figure 2. Mean penile circumference change scores (with standard error bars) during the second interval response window (SIR) following the CS+ and CS- during the preconditioning phase, acquisition phase, and extinction phase for the two conditions Attend and Down-Regulate. Note that during the acquisition phase, the response represents responding to the CS+ plus the US.

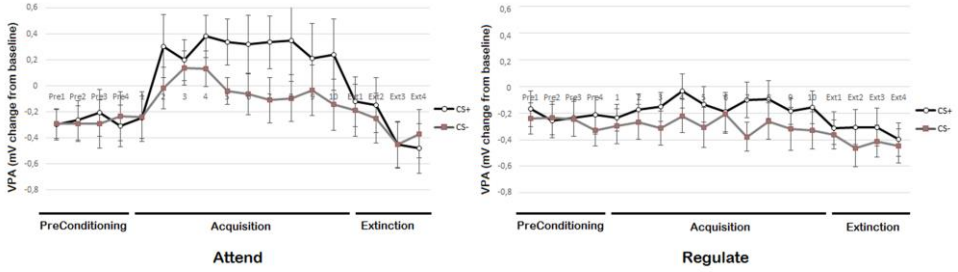

Figure 3. Mean vaginal pulse amplitude (VPA) change scores (with standard error bars) during the second interval response window (SIR) following the CS+ and CS- during the preconditioning phase, acquisition phase, and extinction phase for the two conditions Attend and Down-Regulate. Note that during the acquisition phase, the response represents responding to the CS+ plus the US.

8.3.2. Subjective Measures

Preconditioning phase. The 2 (Stimulus) X 4 (Trial) X 2 (Condition) X 2 (Gender) mixed factor ANOVA to verify equal levels of responding to the CSs revealed no difference in responding following presentation of the CS+ and CS- on affective value and subjective sexual arousal and US expectancy between conditions and sexes, all *p*s > .05.

Extinction phase.

US Expectancy. As can be seen in Figure 4, both conditions showed a robust increase of differential responding towards CS+ and CS- after the acquisition phase, and both conditions showed a decrease in this differential responding over trials. Indeed, the 2 (Stimulus) X 4 (Trial) X 2 (Condition) X 2 (Gender) mixed factor ANOVA revealed a main effect of Stimulus, $F(1, 86) = 227.09$, $p < .01$, $\eta_p^2 = .73$, and a significant interaction effect of Stimulus X Trial $F(2, 212) = 43.97$, $p < .01$, $\eta_p^2 = .34$. No differences were seen between conditions, Stimulus X Condition, $p = .73$, and Stimulus X Trial X Condition, $p = .59$. An interaction of Stimulus X Gender was observed, $F(1, 86) = 8.96$, $p < .01$, $\eta_p^2 = .09$. Women in both conditions showed increased differential responding towards the CS+ and CS-after the acquisition phase compared to men. Analysis of the extinction phase for men and women separately did not reveal significant differences between the two conditions, all *p*s > .18. Analysis of the first extinction trial did not reveal differences in conditioned responding between the two conditions, as reflected by non-significant Stimulus X Condition interactions, men $p = .25$ and women $p = .32$, and neither did analysis of the last extinction trial, men $p = .78$ and women, $p = .15$.

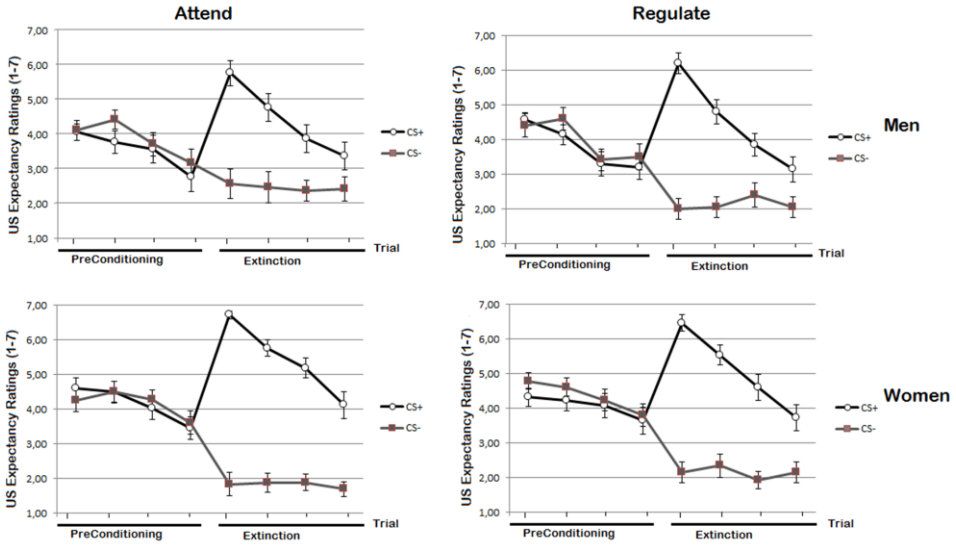

Figure 4. US expectancy ratings (with standard error bars) following the CS+ and CS- during the preconditioning phase and extinction phase for men (top) and women (bottom) in the two conditions Attend (left) and Down-Regulate (right).

Affective Value. As can be seen in Figure 5, men and women differed in conditioned responding after the acquisition phase. The 2 (Stimulus) X 4 (Trial) X 2 (Condition) X 2 (Gender) mixed factor ANOVA revealed a main effect of Stimulus, $F(1, 75) = 27.15$, $p < .01$, $\eta_p^2 = .27$, and an interaction effect of Stimulus X Trial, $F(2, 166) = 4.05$, $p < .02$, $\eta_p^2 = .05$. Also a significant interaction of Stimulus X Trial X Condition X Gender was found, $F(2, 166) = 4,31$, $p < .02$, $\eta_p^2 = .05$. Additional analyses for men and women separately, revealed a significant main effect of Stimulus in men, $F(1, 32) = 11.39$, $p < .01$, $\eta_p^2 = .26$. No interaction of Stimulus X Trial was found, $p = .36$, indicating no

extinction of conditioned responding in men. A significant interaction was found of Stimulus X Trial X Condition, $F(2, 75) = 3.31$, $p < .04$, $\eta_p^2 = .09$, and as can be seen in Figure 5, the Down-Regulate condition demonstrated enhanced extinction of conditioned responding compared to the Attend condition. Analysis of the last extinction trial revealed no significant interaction of Stimulus X Condition, $p = .34$, but a main effect was found of Stimulus, $F(1, 37) = 5.66$, $p < .03$, $\eta_p^2 = .13$, indicating incomplete extinction of conditioned responding with no differences therein between conditions.

For women a main effect of Stimulus was found, $F(1, 43) = 20.01$, $p < .01$, $\eta_p^2 = .32$, and an interaction effect of Stimulus X Trial, $F(2, 88) = 5.06$, $p < .01$, $\eta_p^2 = .11$. Also a main effect of Condition was found, $F(1, 43) = 4.41$, $p = .04$, $\eta_p^2 = .09$. As can be seen in Figure 5, compared to the Attend condition, women in the Down-Regulate condition demonstrated overall higher responses towards the CS+ and CS- in the extinction phase. No interaction effects of Stimulus X Condition and Stimulus X Trial X Condition were seen, ps > .33. Analysis of the first extinction trial for women revealed no significant interaction of Stimulus X Condition, $p = .33$. Analysis of the last extinction trial did also not reveal differences in conditioned differential responding towards the CS+ and CS- between the two conditions, $p = .60$. On this last trial there was still a main effect of Stimulus, $F(1, 50) = 13.32$, $p < .01$, $\eta_p^2 = .21$, indicating no extinction of conditioned differential responding.

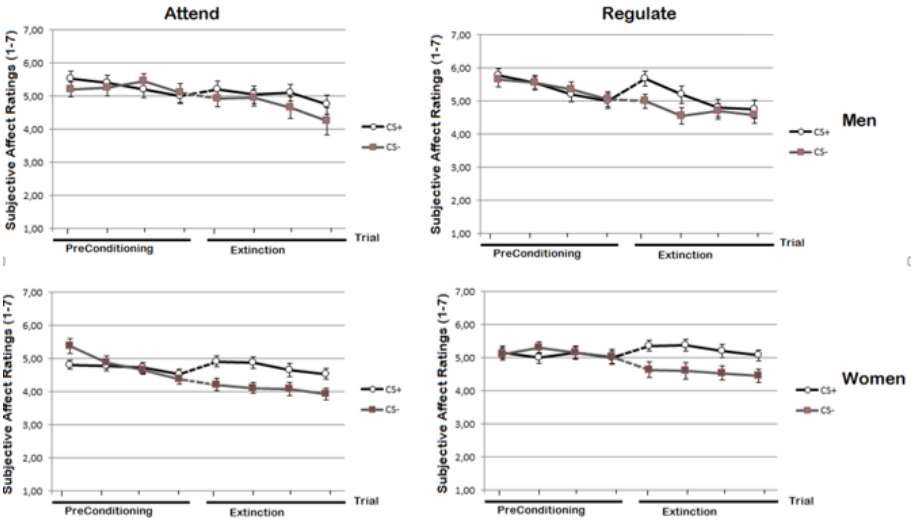

Figure 5. Subjective affect ratings (with standard error bars) following the CS+ and CS- during the preconditioning phase and extinction phase for men (top) and women (bottom) in the two conditions Attend (left) and Down-Regulate (right).

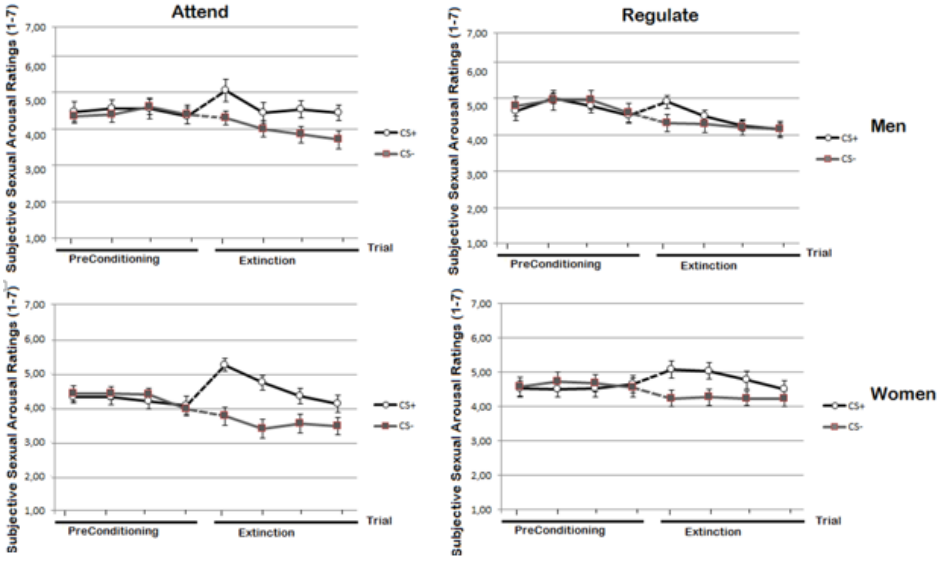

Figure 6. Ratings (with standard error bars) of subjective sexual arousal following the CS+ and CS- during the preconditioning phase and extinction phase for men (top) and women (bottom) in the two conditions Attend (left) and Down-Regulate (right).

Subjective Sexual Arousal. Figure 6 shows increased ratings of subjective sexual arousal towards the CS+ on the first trials of the extinction phase in men and women. The 2 (Stimulus) X 4 (Trial) X 2 (Condition) X 2 (Gender) mixed factor ANOVA revealed a significant main effect of Stimulus, $F(1, 81) = 23.22$, $p < .01$, $\eta_p^2 = .23$, and an interaction effect of Stimulus X Trial, $F(3, 213) = 8.05$, $p < .01$, $\eta_p^2 = .09$, and Stimulus X Trial X Condition X Gender, $F(3, 213) = 2.85$, $p < .05$, $\eta_p^2 = .03$. Therefore, additional analyses were conducted for men and women separately. In men, no significant interaction of Stimulus X Condition was found, $p = .12$, nor of Stimulus X Trial X Condition, $p = .15$. However, analysis of the last two extinction trials revealed a significant interaction of Stimulus X Condition, $F(1, 37) = 4.34$, $p < .05$, $\eta_p^2 = .11$. Analysis of the last extinction trial also revealed a significant Stimulus X Condition interaction, $F(1, 38) = 5.12$, $p < .03$, $\eta_p^2 = .12$.

For women, analysis of the extinction phase revealed a significant Stimulus X Trial interaction effect, $F(2, 106) = 2.91$, $p < .01$, $\eta_p^2 = .15$. No significant interaction effects of Stimulus X Condition, $p = .38$, or Stimulus X Trial X Condition, $p = .19$, were observed. A main effect of Condition was seen, $F(1, 45) = 4.16$, $p < .05$, $\eta_p^2 = .09$. As can be seen in Figure 6, women in the Down-Regulate condition demonstrated overall higher ratings of subjective sexual arousal towards both CS+ and CS- in the extinction phase, as compared with women in the Attend condition. Additional analysis of only the first extinction trial for men and women separately did not reveal a significant Stimulus X Condition interaction, men $p = .55$, women $p = .13$. Analysis of the last preconditioning trial and the first extinction trial for only CS+ responses, revealed a main effect of Stimulus in men, $F(1, 37) = 8.39$, $p < .01$, $\eta_p^2 = .19$, and

women, $F(1, 47) = 24.97$, $p < .01$, $\eta_p^2 = .35$, and a significant interaction effect of Stimulus X Condition in women, $F(1, 47) = 5.11$, $p < .03$, $\eta_p^2 = .10$. The Down-Regulate condition demonstrated attenuated responding towards the CS+ compared to women in the Attend condition. Analysis of the last extinction trial did not reveal a significant Stimulus X Condition interaction, $p = .28$, but a significant main effect was observed of Stimulus, $F(1, 50) = 6.84$, $p < .02$, $\eta_p^2 = .12$, indicating there was still differential conditioned responding on the last extinction trial, with no differences therein between conditions.

8.3.3. Approach and Avoidance Tendencies

The preconditioning AAT bias scores were analysed with a mixed factor ANOVA with Gender and Condition as between-subjects factors and Image as within-subject factor with three levels (CS+, CS-, and neutral pictures). In line with the expectations, no interaction effect of Image and Condition was found, $p = .45$. Men and women also did not seem to behave differently in approach and avoidance tendencies towards the stimuli before the conditioning procedure, as reflected by the non-significant Image X Gender interaction, $p = .60$.

The mixed factor ANOVA with Gender and Condition as between-subjects factors and Image as within-subject factor with three levels (CS+, CS-, and neutral pictures) and Trial as within-subjects factor with two levels (preconditioning and post conditioning), of the AAT preconditioning and AAT post conditioning bias scores, revealed an interaction of Image X Trial X Gender, $F(1, 127) = 22.07$, $p < .01$, $\eta_p^2 = .20$. No Image X Trial X Condition effect was observed, $p = .37$. Analysis for men and women separately, revealed

no significant results for men, all *p*s> .31, whereas for women a significant Image X Trial interaction was found, $F(2, 81) = 61.52$, $p < .01$, $\eta_p^2 = .55$.

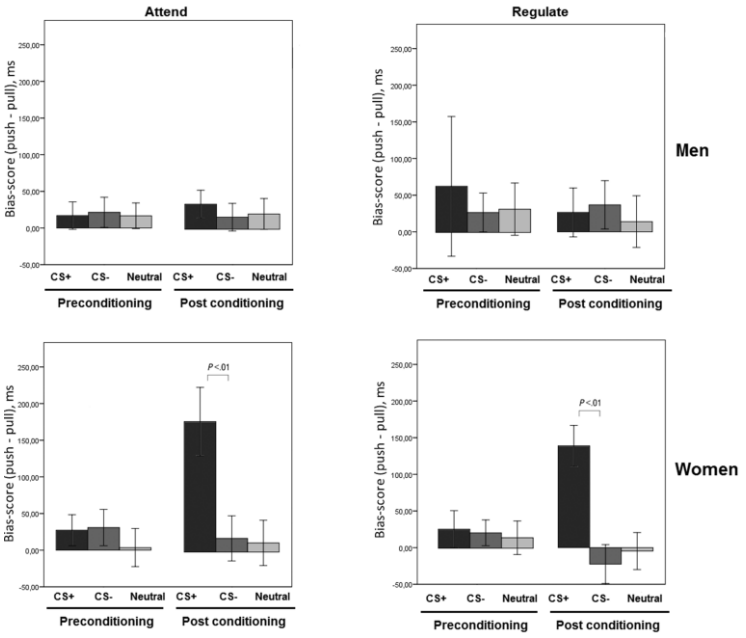

Figure 7. Approach Avoidance Task (AAT) bias scores for CS+, CS-, and neutral images in men (above) and women in the Attend and Down-Regulate condition (ms with standard error bars), preconditioning and post conditioning. A positive score indicates faster reaction times on approach (pull) trials compared to avoid (push) trials.

Analysis of only the post conditioning AAT scores demonstrated a significant main effect of Image, $F(2, 137) = 55.97$, $p < .01$, $\eta_p^2 = .39$, and of Image X Gender, $F(2, 137) = 52.64$, $p < .01$, $\eta_p^2 = .37$. No significant interaction of Image X Condition was found, $p = .61$. Analysis of post conditioning bias scores for men and women separately, demonstrated a main effect of Image in women, $F(1, 68) = 91.46$, $p < .01$, $\eta_p^2 = .64$, whereas in men it did not, $p = .41$. For

women, also a main effect of Condition was seen, $F(1, 51)= 4.19$, $p< .05$, $\eta_p^2 = .08$. Compared to women in the Attend condition, women in the Down-Regulate condition had attenuated approach biases towards all stimuli, as can be seen in Figure 7.

			Affective Value	Subjective Sexual Arousal	US Expectancy	Condtioned Genital Response SIR	Condtioned Genital Response TIR	Bias Score CS+	Bias Score CS-
Men	**Attend**	Affective Value		.67**	.45	-.19	-.18	-.34	-.39
		Subjective Sexual Arousal	.67**		.45*	.14	.14	-.32	-.12
		US Expectancy	.45	.45*		.14	.13	.01	.12
		Conditioned Genital Response SIR	-.19	.14	.14		.99**	.07	.31
		Conditioned Genital Response TIR	-.18	-.14	.13	.99**		.06	.30
		Bias Score CS+	-.34	-.32	.01	.07	.06		.49*
		Bias Score CS-	-.39	-.12	.12	.31	.30	.49*	.18
	Regulate	Affective Value		.49*	.11	.30	.18	-.17	.18
		Subjective Sexual Arousal	.49*		.12	.14	-.21	.14	.13
		US Expectancy	.11	.12		-.01	-.13	.42	.13
		Conditioned Genital Response SIR	.30	.14	-.01		.73**	-.13	-.05
		Conditioned Genital Response TIR	.18	-.21	-.13	.73**		.01	.07
		Bias Score CS+	-.17	.14	.42	-.13	.01		.60**
		Bias Score CS-	.18	.13	.13	-.05	.07	.60**	
Women	**Attend**	Affective Value		.71**	.38	-.40	-.31	.10	-.17
		Subjective Sexual Arousal	.71**		.40	-.14	-.20	-.16	-.31
		US Expectancy	.38	.40		-.11	-.15	-.15	.05
		Conditioned Genital Response SIR	-.40	-.14	-.11		.48*	-.25	-.14
		Conditioned Genital Response TIR	-.31	-.20	-.15	.48*		.15	.08
		Bias Score CS+	.10	-.16	-.15	-.25	.15		.20
		Bias Score CS-	-.17	-.31	.05	-.14	.08	.20	

		Affective Value	Subjective Sexual Arousal	US Expectancy	Condtioned Genital Response SIR	Condtioned Genital Response TIR	Bias Score CS+	Bias Score CS-
Regulate	Affective Value		.32	.13	-.18	.22	-.20	.11
	Subjective Sexual Arousal	.32		.39	.04	.07	.01	-.11
	US Expectancy	.13	.39		-.26	.07	-.32	.18
	Conditioned Genital Response SIR	-.18	.04	-.26		-.35	.26	.16
	Conditioned Genital Response TIR	.22	.07	.07	-.35		-.33	-.23
	Bias Score CS+	-.20	.01	-.32	.26	-.33		.12
	Bias Score CS-	.11	-.11	.18	.16	-.23	.12	

Table 2. Correlations between conditioned genital response, conditioned affective change, conditioned subjective sexual arousal, conditioned US expectancy and conditioned approach and avoidance tendencies towards the CS+ and CS- for men and women, in the Attend and Regulate condition. Notes: ** Correlation is significant at the .01 level (2-tailed), * Correlation is significant at the .05 level (2-tailed).

8.3.4. Correlations between Conditioned Responses

To investigate relationships between conditioned responses additional correlational analyses were conducted. We expected that the strength of the conditioned genital response would be positively related to the amount of change in subjective affect and subjective arousal and US expectancy. In addition, it was expected that the strength of the conditioned genital response would be positively related to the CS+ bias score. To investigate these relationships, for genital responses on SIR and TIR and ratings of affect, and subjective sexual arousal and US expectancy, the difference between the response to the CS+ and the CS− during the first trial in the extinction phase was calculated by subtracting the response to the CS− from the response to the CS+. Pearson product-moment correlations between genital difference scores, affect difference score, subjective sexual arousal difference score, and US expectancy difference scores, were calculated (see Table 2). Table 2 shows that there were no significant correlations between the strength of the conditioned genital response and conditioned subjective and behavioural measures in men and women, in both the *Attend* and *Regulate* condition

8.4. Discussion

The present study is the first that included men and women in the same experimental conditioning design on emotion regulation, and it is remarkable that a gender difference in subjective and behavioural sexual response was observed. First, the deployment of a cognitive emotion down-regulation strategy effectively enhanced extinction of conditioned affective value and subjective sexual arousal in men as compared to men in the *Attend* condition. This difference in enhanced extinction of conditioned subjective sexual arousal between the two conditions in men is substantial given the found effect sizes. Intriguingly, in women no evidence was found that cognitive down-regulation results in enhanced extinction of conditioned differential affect value or

subjective sexual arousal towards the CS+ and CS-. Surprisingly, and contrary to the expectations, women in the Down-Regulate condition demonstrated overall higher ratings of affective value and subjective sexual arousal towards the CS+ and CS- in the extinction phase, compared with women in the Attend condition. Second, compared with an attend stimulus strategy, cognitive down-regulation strategies resulted in attenuated approach tendencies towards conditioned stimuli that predicted potential sexual reward in women, but not in men. Although men demonstrated more robust conditioned genital response, strong approach tendencies were not observed. However, such tendencies need not per se translate into overt behaviour, since although emotions involve an automatic tendency to act, emotional impulses can be regulated by cognitive evaluation processes operating under cognitive control (Frijda. 2010).

It is crucial to mention that not all hypotheses were confirmed. First, no evidence for cognitive emotion down-regulation strategies to affect acquisition of conditioned genital response in men and women was found. Additionally, compared with an attend stimulus strategy, cognitive down-regulation strategies did not result in decreased conditioned genital sexual arousal, or subjective affect and sexual arousal in both sexes. Lastly, it seems US expectancy in men and women is not affected at all by cognitive emotion down-regulation strategies. Results also showed that no significant correlations existed between the strength of the conditioned genital, subjective and behavioural response, with no differences therein between men and women.

It is tempting to speculate that women may indeed use less efficient cognitive strategies compared to men (Whittle et al., 2011). Results from the exit interview also revealed that women experienced more difficulties with the deployment of the cognitive down-regulatory strategy. It is postulated that in primary emotions, which arise as a result of processing innately significant environmental stimuli, like sexual cues, the limbic structures are primary involved. Secondary emotions -that are evoked by environmental and

experiential stimuli that have acquired significance through learning- are thought to involve the additional participation of the prefrontal and somatosensory cortices, which also function to modulate limbic system activation (Damasio, 1994; LeDoux, 2000). Research revealed that men rely more on prefrontal and somatosensory cortices (especially the dorsolateral prefrontal cortex) during emotion regulation, whereas women rely more on limbic regions including the left hippocampus, the left amygdala and insular cortex (Kong et al., 2014; Whittle, 2011). The observed greater limbic activation in women (Whittle, 2011) might suggest that their emotional perception may be more of the primary than the secondary type, and this may facilitate quicker and more accurate perception. In men, emotional perception may be more impacted upon by regulatory and associative processes, leading to a greater ability to regulate emotions, including sexual arousal (Whittle, 2011). Research on the regulation of sexual arousal in men showed that experienced sexual arousal is associated with activation in "limbic" and paralimbic structures, whereas inhibition of sexual arousal is associated with activation of the right superior frontal gyrus and right anterior cingulate gyrus (Beauregard, Lévesque & Bourgouin, 2001). Intriguingly, no activation was found in limbic areas during inhibition of sexual arousal. Unfortunately, at present no imaging studies have been conducted that have investigated down- or up regulation of sexual arousal in women. However, an imaging study by Klucken et al. (2014) revealed that the Val^{158}Met-ploymorphism in the Catechol-O-Methyl-Transferase (COMT) is associated with the alteration of neural processes of appetitive conditioning. Individuals who carried the Val/Val-allele demonstrated increased hemodynamic responses in the amygdala compared with the Met/Met-allele group in a differential conditioning paradigm. Although participants were not explicitly instructed to use emotion regulation strategies in this study, in Met/Met-allele carriers an increased effective amygdala-ventromedial prefrontal cortex connectivity was found, and this could be

regarded as a marker for altered emotion regulation during conditioning. These findings emphasize the importance of genetic variations on appetitive conditioning, and subsequent increased vulnerability for addiction disorders or maladaptive sexual behaviours.

Given the problems in comparing genital responses of men and women directly, and possible differences between sexes with regard to responses to specific types of stimulus materials, it is far too early to infer that women indeed are less efficient in down regulation of positive (sexual) emotions than men. In addition, the effect of the emotional down-regulatory strategy in the present study is relative to the other (*Attend*) strategy with which it is compared and does therefore not reflect the complexities of the emotion regulation repertoire (Aldao, 2013). Future studies should therefore investigate if the found gender differences are also seen making use of multiple cognitive down-regulatory strategies (like cognitive reappraisal, or concentrating on the neutral and asexual aspects of the CSs). In addition, another limitation of the present study is the absence of a between subjects (unpaired) control group. Without such a control group it is difficult to determine whether and what learning has occurred. At present it is unclear if the differential response towards the CS+ and CS− was due to conditioning or to pseudo conditioning. The possibility of sensitization of sexual arousal would translate into increased genital responses across trials, and not in differential responding towards the CS+ and CS− per se (Domjan, 2010; Hoffmann et al., 2014). Therefore, making use of such a control group in future research is desirable.

It is suggested that antecedent-focused strategies like attentional deployment are more effective than response-focused strategies (Gross & Thompson, 2007). In the meta-analysis by Webb, Miles and Sheeran (2012), passive distraction strategies (where participants are provided with materials or a task that is unrelated to the emotion or emotional stimulus) had small effects on emotional outcomes, whereas active distraction strategies (where the

emphasis is on participants to bring to mind something unrelated to the emotion or emotional stimulus to serve as a distraction) had small-to-medium-sized effects. It was postulated that explicitly instructing participants to think about something unrelated to the emotion is more effective than simply providing a distracting task. More research is needed since research on the regulation of positive emotions like sexual arousal is extremely scarce. Moreover, the majority of the empirical investigations on emotion regulation (Aldao. 2013), including the present study, have examined processes in healthy individuals, and only little attention has been devoted to how those processes might differ as a function of variability in psychopathology status. As it is suggested that personality facets and dispositional and state-level psychological processes influence emotion regulatory processes (Aldao, 2013), an important venue for future research is the tailoring of the emotion regulation strategies to the individual patient.

The present study is the first that found conditioned genital response in men making use of a tactile US. In line with Brom et al. (2014b) men showed a smaller penile circumference in response to the CS+ during the acquisition phase and when vibrostimulation no longer was applied. Former research on automatic and controlled cognitive processing of sexual stimuli also found male genital responses to be opposite to the predictions: genital responses towards sexually primed targets were lower than responses to neutrally primed targets (Janssen et al., 2000). Those results were explained by physiological processes of penile erection. During the initial phases of erectile response, the penis undergoes an increase in length, and this is associated with a simultaneous decrease in circumference. Therefore, the physiology of penile erection may also account for the results found in the present study, with the smaller penile circumference in response to the CS+ reflecting the initial stage of penile erection.

Our results suggest that in the treatment of problematic strong sexual arousal and appetite, cognitive strategies in the processing of conditioned sexual stimuli may be helpful. It is important to mention that in the present study, instructions to regulate were given also during the acquisition phase, which would not reflect how regulation instructions would be offered in a clinical setting. In the treatment of problematic sexual arousal, clients are taught regulation strategies after having developed problematic behaviours via maladaptive conditioning. Nevertheless, learning to obtain effective emotion regulation strategies in circumstances in which sexual stimuli cannot be avoided may be useful to diminish undesirable feelings of sexual arousal and desire and to exert control over sexual behaviour. Therefore, future studies should incorporate clinical samples, like individuals with hypersexuality or deviant sexual preferences that manifest perturbed motivation. Second, as mentioned before, future conditioning studies should also make use of a design in which the instructions to regulate are given only after acquisition has occurred, herewith resembling to the clinical setting more closely. Still, results from the present study suggest that cognitive emotion regulatory strategies may be more effective in controlling unwanted sexual feelings than extinction by cue-exposure treatment alone, as research from our lab has shown that diminished sexual responses can return (Brom et al., 2014b). On the other hand, in case of hyposexuality, increasing sexual arousal by making use of up-regulatory cognitive strategies may be effective. Therefore, future research should investigate if cognitive up-regulatory strategies can indeed be helpful in increasing sexual arousal elicited by conditioned sexual cues.

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Chapter 9

The influence of Emotion Up-Regulation on The Expectation of Sexual Reward

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Abstract

Emotion regulation research has shown successful altering of unwanted aversive emotional reactions. Cognitive strategies can also down-regulate expectations of reward arising from conditioned stimuli, including sexual stimuli. However, little is known about whether such strategies can also efficiently up-regulate expectations of sexual reward arising from conditioned stimuli, and possible gender differences therein. In the present study it was examined whether a cognitive up-regulatory strategy could successfully up-regulate sexual arousal elicited by sexual reward-conditioned cues in men and women. Men (n= 40) and women (n= 53) participated in a study using a differential conditioning paradigm, with genital vibrostimulation as unconditioned stimulus (US) and sexually relevant pictures as conditional stimuli (CSs). Penile circumference and vaginal pulse amplitude were assessed and ratings of US expectancy, affective value and sexual arousal value were obtained. Also a stimulus response compatibility task was included to assess automatic approach and avoidance tendencies. Evidence was found for emotion up-regulation to increase genital arousal response in the acquisition phase in both sexes, and to enhance resistance to extinction of conditioned genital responding in women. In men, the emotion up-regulatory strategy resulted in increased conditioned positive affect. The findings support that top-down modulation may indeed influence conditioned sexual responses. This knowledge may have implications for treating disturbances in sexual appetitive responses, such as low sexual arousal and desire.

9.1. Introduction

According to incentive motivation models, aetiology and maintenance of low sexual arousal and desire, such as in Female Sexual Interest/Arousal Disorder (Diagnostic and Statistical Manual of Mental Disorders, DSM-5), can be explained from a classical conditioning perspective (Ågmo, 1999; Bindra, 1974; Brom et al., 2014a; Laan & Both, 2008). Learning about sexual cues may encompass learning of positive expectations of pleasure and sexual reward, but may also include the learning of negative expectations (Ågmo, 1999; Brom et al., 2014). External stimuli that can elicit sexual motivational responses are called sexual incentive stimuli (Ågmo, 1999; Singer & Toates, 1987). The motivational valence of incentive stimuli can be unconditioned (primary) or conditioned (secondary) as a result of associative leaning (Di Chiara, 1995). In associative learning processes like classical conditioning, a neutral stimulus (NS) is repeatedly paired with an unconditioned stimulus (US) (Pavlov, 1927), and eventually the NS is able to elicit the same reaction as the US (Bindra, 1974; Pavlov, 1927). The NS is now called the conditioned stimulus (CS) and the reaction to the CS is called the conditioned response (CR). It is suggested that the contingent pairing of negative emotional experiences (e.g. sexual assault or repeated experiences with painful coitus) with stimuli that used to have sexual incentive value, may result in less attraction or even aversion to these incentives (Both et al., 2008; Brom et al., 2015a). This lack of a positive sexual learning history, or even a more negative learning history, may result in a limited number and/or in limited strength of potential sexual incentives that can activate the sexual response system, and subsequently in reduced or lacking feelings of sexual desire and arousal (often in the absence of disturbed genital response) (Basson, et al., 2003; Both, Everaerd & Laan, 2007; Both, Laan & Schultz, 2010; Brauer et al., 2012; Everaerd & Laan, 1995).

Although there is limited empirical support, cognitive behavioural therapy (CBT) based on associative learning principles has emerged as the psychological treatment of choice for disorders in sexual interest and desire (Basson, 2005; Both, Laan & Schultz, 2010; Laan & Both, 2008; Trudel, Marchand, Ravart, 2001). Core components of CBT are cognitive techniques such as cognitive restructuring of negative and sexually inhibiting thoughts, and behavioural techniques such as sex therapeutic exercises to (re)create different, more varied, or prolonged sexual stimulation to enhance sexually pleasurable experiences. It is thought that the interaction with pleasurable sexual stimuli and events desensitizes possible negative associations and facilitates sexual response acquisition and maintenance, and that memories of positive sexual experiences result in expectations of sexual reward, which may subsequently enhance sexual interest and arousal (Basson, 2005; Both, Laan & Schultz, 2010; Laan & Both, 2008). It is likely that cognitive and behavioural processes interact during CBT. Experiences during sex therapeutic exercises may change cognitions, and cognitive restructuring, in turn, may facilitate acquisition of pleasurable sexual associations. The term emotion regulation (ER) signifies any process that serves to initiate, inhibit or modulate (e.g. cognitively re-evaluate) emotional feelings or behaviour (Aldao, 2013; Gross, 2002; Gross & Thompson, 2007). The ER techniques 'reappraisal' (i.e. cognitive change, yielding an altered interpretation of an emotional situation) and attentional focus (decreasing or increasing attention to the emotional and physical impact of the stimulus) have been proposed to be effective regulatory strategies because their influence begins at an early stage of emotion generation, before emotional responses have fully unfolded (Ochsner & Gross, 2005). Insight in the mechanisms of these cognition-emotion interactions can help in the development of effective CBT interventions. In the present study it was investigated whether deployment of an emotion up regulatory strategy can facilitate the acquisition of conditioned sexual responses. The present study

created a laboratory analogue of CBT by applying a key feature of cognitive restructuring (i.e. cognitive up-regulation of sexual arousal response evoked by US/CS by means of reappraisal and attentional focus) to the laboratory analogue of basic sexual reward learning (i.e. classical conditioning).

There is growing evidence that cognitive strategies like attentional deployment can down-regulate expectations of reward arising from conditioned stimuli (Delgado, Gillis & Phelps, 2008), including sexual conditioned stimuli (Brom et al., 2015b). However, less is known about the efficacy of up-regulatory strategies in sexual arousal. Nevertheless, studies on positive emotion up-regulation have demonstrated that reappraisal of positive images (i.e. up-regulation of positive affect) influenced the early stage of emotional response, and was associated with adaptive hemodynamic profiles both during anticipation and during viewing of affective images depending on their valence and the regulatory goal (Pavlov et al., 2014). In addition, in another study (Moholy et al., 2015), before each sexual film, participants were instructed to increase their sexual arousal, decrease their sexual arousal or respond as usual. They found that on average, participants performed the task as instructed. However, individuals with higher sexual desire for a partner exhibited less change in their sexual arousal to regulation instructions. Moreover, in a neuroimaging study from our lab (*in preparation*) 40 healthy male participants had to increase (´Up´), decrease (´Down´) or maintain ('Equal') their sexual arousal response evoked by sexual explicit pictures inside a MRI-scanner. Down-regulation of sexual arousal activated prefrontal regions, while up-regulation activated reward-related structures such as the nucleus accumbens and amygdala. These studies suggest that men and women can effectively enhance sexual arousal levels making use of up-regulatory strategies. However, despite its presumed importance, research on the regulation of reward expectations elicited by sexual conditioned stimuli is lacking in the literature. In addition, it is unclear whether men and women are equally prone to

conditioning of sexual response and whether sex differences do exist in the emotion regulation of positive emotions, like sexual arousal (Brom et al., 2014; 2015a,b; Domjan, 2005; Hoffmann, Janssen & Turner, 2004; Klucken et al., 2009; Moholy et al., 2015; Pfaus, Kippin & Centeno, 2001). However, regarding possible gender differences in emotion regulation, the general assertion is that women tend to use more emotion-focused strategies, while men are thought to use more effective cognitive (rational) cognitive strategies (Whittle et al., 2011). To be specific, in their review of neuroimaging research, Whittle et al. (2011) suggests that women may recruit different brain regions compared to men during emotion perception. In general this seems to be associated with greater levels of limbic/subcortical and temporal activation in women compared to men, and greater levels of frontal and parietal activation in men compared to women. Moreover, the authors suggest that men and women use different strategies to down-regulate negative emotions, and that these strategies might be mediated by different neural circuitry. Men seem to engage in automatic or unconscious emotion regulation when exposed to emotional stimuli, which may result from greater integration of cognitive and emotional neural circuits. However, most of these results on gender differences in ER relate to the regulation of particularly negative emotions (Mak et al., 2009; McRae et al., 2008; Gross, 2007; Whittle et al., 2011).

A recent study demonstrated that women may indeed use less effective cognitive strategies compared to men also in the regulation of positive emotions (Brom et al., 2015b). Making use of a differential sexual conditioning paradigm, evidence was found for the deployment of a cognitive emotion down-regulation strategy to effectively enhance extinction of conditioned affective value and subjective sexual arousal in men, whereas this cognitive strategy in women resulted in overall higher ratings of affective value and subjective sexual arousal towards the CS+ and CS- in the extinction phase compared to a control condition. Compared to men, women also reported

experiencing more difficulties with the deployment of the cognitive down-regulatory strategy. The fact that this study only investigated the influence of emotion down-regulation on conditioned sexual response (Brom et al., 2015b), combined with the lack of studies on sex differences in positive emotion up-regulation, point to the importance for further investigation of possible gender differences in sexual learning and cognitive (up-)regulation thereof.

As a result of classical conditioning, a CS cannot only become a signal of upcoming reward, it can also acquire the hedonic valence of the US. This form of learning involves the transfer of affective value to an initially neutral stimulus as a result of its contingent presentation with (dis)liked stimuli, and is called evaluative conditioning (De Houwer, Thomas & Baeyens, 2001; Hermans et al., 2002). While in classical conditioning the CS elicits a US expectancy and CR (i.e. signal learning), in evaluative learning it is thought that the CS automatically comes to evoke the representation of the US (Diaz, Ruiz & Baeyens, 2005). Research has demonstrated that evaluative conditioning is more resistant to extinction than expectancy learning (i.e. autonomic physiological responses and ratings of US expectancy) (Baeyens et al., 1992; Brom et al., 2015a; *submitted*; De Houwer, Thomas & Baeyens, 2001), and is associated with reinstatement of conditioned responding (Dirkx et al., 2004; Hermans et al., 2005) which makes evaluative conditioning particularly relevant for the long term outcome of CBT.

The present study is the first to investigate whether a cognitive up-regulatory strategy can efficiently increase sexual arousal elicited by sexual reward-conditioned cues in healthy men and women. Applying a differential conditioning paradigm, it was predicted that participants in two conditions (the control *Attend* condition and the experimental *Up-Regulate* condition) would show conditioned genital and subjective sexual responding to the CS that was paired with the US (the CS+), which was expected to gradually decrease during

extinction trials. It was predicted that an emotion up-regulatory strategy should increase sexual arousal elicited by the sexual reward-conditioned cue compared to the control condition, in men and women, in both the acquisition and extinction phases. Furthermore, it was predicted that deployment of the emotion up-regulation strategy would affect evaluative learning, as measured by ratings of subjective affective value and sexual arousal value, rather than expectancy learning, as measured by physiological genital sexual response and ratings of US expectancy (Blechert et al., 2015). Since subjective ratings are susceptible to demand characteristics, in addition a task was included to assess implicit approach and avoidance tendencies towards the CSs (Cousijn, Goudriaan & Wiers, 2011). We assumed that after the conditioning procedure, participants should be faster when instructed to approach the CS+ and avoid from the CS- than when instructed to avoid the CS+ and approach the CS-, and that an emotion up-regulation strategy should increase these responses.

9.2. Methods

9.2.1. Participants

Research participants were 40 men and 53 women. Participants were paid (€30,-) for their participation and were recruited using posted advertisements. The advertisement stated that the focus of the study would be on the relationship between erotic stimulation and sexual arousal. Inclusion criteria were: age between 18 and 45 years and a heterosexual orientation. Exclusion criteria were: sexual problems, a Diagnostic and Statistical Manual of Mental Disorders (DSM-5) diagnosis of an affective or psychotic disorder or abusive drug use, pregnancy or breastfeeding, and a medical illness or use of medication that could interfere with sexual response. Written informed consent was obtained

from all participants. The study was approved by the Ethical Committee of the Medical Centre.

9.2.2. Design and conditioning procedure

Participants were randomly assigned to one of the two conditions: *Up-Regulate* or *Attend*, with restriction that conditions matched on sex as close as possible. During conditioning, one stimulus (the CS+) was followed by the genital vibrostimulation (US) during the acquisition phase, whereas the other stimulus (CS-) was never followed by genital vibrostimulation. For a schematic overview of the procedure see Figure 1. In the preconditioning phase, participants saw four nonreinforced presentations of the CS+ and four presentations of the CS-, for 9s each. Subsequently, in the acquisition phase the CS+ and CS- were presented 10 times each and the CS+ was always followed by the US. In the extinction phase, consisting of 4 trials, the CS+ was no longer followed by the US. Prior to CS presentation, in the acquisition- and extinction phases participants were presented with a written cue (*Attend* or *Up-regulate*) on screen for 2s that reminded participants to either attend to- or up-regulate (i.e. increase) sexual arousal when seeing their CS+. All phases were presented without interruption. Genital response was measured continuously during resting baseline, preconditioning, acquisition, and extinction phases. There were two random CS orders for each phase (that was counterbalanced across participants), with the restriction of only two successive presentations of each CS. During the whole procedure inter-trial intervals (ITIs) were 20, 25, or 30s. The order of the length of the ITI was random, with the restriction of only two successive lengths.

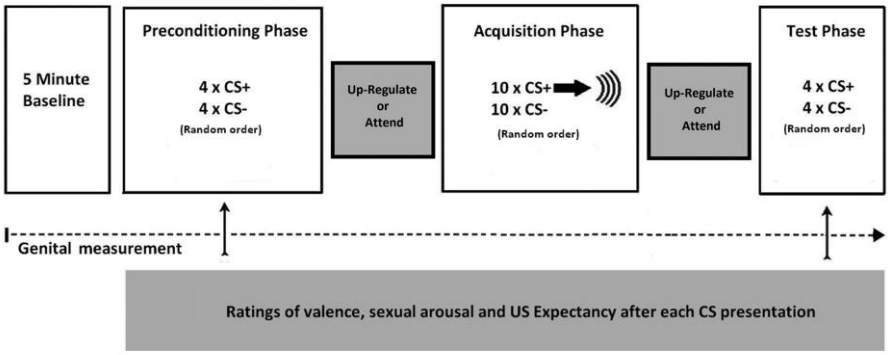

Figure 1. Schematic representation of the experimental procedure in both conditions. In the acquisition and extinction phase, before every CS presentation a written cue was presented: participants in the Up-Regulate condition received the instruction Up-Regulate whereas participants in the control condition received the written cue Attend prior to each CSs. Assignment of the colour of the pictures (blue or yellow) as CS+ and CS− was counterbalanced across participants and conditions.

9.2.3. Materials, Apparatus, and Recording

Stimulus materials. Two identical pictures served as CSs, and portrayed a torso of an individual of the opposite sex (a female torso with clothed breasts and genitals, or a men's exposed chest and clothed genitals), with the colour of the underwear in the picture (Blue or Yellow) being the only difference (Brom et al., 2015b). The CSs were shown in the middle of a computer monitor, approximately 1.5 m in front of the participant. The size of the presented pictures was 14 X 21 cm. Assignment of the pictures as CS+ and CS- was counterbalanced across participants and conditions. Stimuli and cues were presented by using E-prime 2.0 Software (Psychology Software Tools Inc., Sharpsburg, USA).

Written instructions. Prior to each trial in the acquisition and extinction phases, participants received a written cue on screen. In the *Attend* condition participants received the written cue *Attend*, and they were instructed to '*just pay attention'* to the CSs when they were presented this cue. In contrast, in the *Up-Regulate* condition participants were presented with the cue '*Up-Regulate'* in the acquisition and extinction phases, and were instructed that when this cue appeared on the monitor, they should increase any experienced/felt sexual response and arousal the CSs might elicit. Specifically, they were instructed to: *'concentrate on the bodily sensations you may feel such as genital sensations, changes in heartbeat, or tingles in your body, and increase any positive feelings you may experience such as sexual arousal and excitement when receiving the genital vibrostimulation and seeing the CS+. For instance, you could imagine as if you are engaged in actual sexual activities*.' Participants were aware of the contingencies and well-practiced the instructions before commencing the experimental session. Participants were asked to verbalize their strategy when being presented with the written cues *Attend* and *Up-Regulate* to assure that they were following the instructions they were given.

Genital vibrostimulation (US). Genital vibrostimulation was provided 8s following the start of the CS+ for 2s. For men, the US was administered by means of a ring-shaped vibrator. They were instructed to place the vibrator just below the coronal ridge (Brom et al., 2015b) and to position the vibrator as *most sexually stimulating*. For women, a small hands-off vibrator (2 cm diameter) was used (Laan & van Lunsen, 2002). The vibrator was placed on the clitoris using lycra underwear that had an opening for the vaginal plethysmograph. Women were also instructed to position the vibrator as *most sexually stimulating*.

9.2.4. Main Outcome Measures

Male genital sexual arousal

An indium/gallium-in-rubber penile gauge assessed changes in penile circumference (Bancroft, Jones & Pullan, 1966). The gauges were calibrated before each laboratory session using a set of calibrated rings (Janssen, Prause & Geer, 2007). The penile gauge was positioned two-thirds of the way down the shaft of the penis toward the base. Changes in electrical output caused by expansion of the gauge were recorded by a continuous DC signal. The Indium-Gallium penile gauges were disinfected after each use, according to Sekusept plus disinfection procedure (MedCaT B.V.). Sekusept plus contains Glucoprotamine, which action spectrum covers bacteria including mycobacteria, fungi and viruses (e.g. Human Papillomavirus [HPV]) (MedCaT B.V.).

Women's genital arousal

Vaginal photoplethysmography assessed vaginal pulse amplitude (VPA) (Laan, Everaerd & Evers, 1995). Depth of the probe and orientation of the light emitting diode were controlled by a device (a 6- X 2-cm plate) attached to the cable. The vaginal photoplethysmograph was disinfected by means of a plasma sterilization procedure between uses. Plasma sterilization is a highly effective method for the complete removal of all organic (and certain in-organic) materials (De Geyter & Morent, 2012). Research provides support for the notion that VPA is a reliable measure specific to sexual arousal (Laan, Everaerd & Evers, 1995; Suschinsky, Lalumière & Chivers, 2009).

Subjective ratings

Ratings of affective value, sexual arousal and US expectancy were collected during the preconditioning- and extinction phases. Participants were first asked

to rate, after each CS presentation, the affective value of the CSs by answering the question "*What kind of feeling does this picture evoke in you*?" The question could be answered on a seven-point Likert scale on a keyboard that varied from *very negative* to *very positive*. Then, sexual arousal value was rated by answering the question "*How sexually arousing is this picture to you*?" The question could be answered on a seven-point scale that varied from *not sexually arousing at all* to *very sexually arousing*. Then, participants were required to rate the expectancy of a vibration following the presentation of each CS on a seven-point scale by answering the question "*To what extent did you expect a vibration after this picture*"? The scale consisted of seven points labelled from '*certainly no vibration'* through '*certainly a vibration'*. The questions were presented at the monitor 1 second following the end of picture presentation.

9.2.5. Other Measures

Approach Avoidance Task (AAT (Cousijn, Goudriaan & Wiers, 2011), E-prime 2.0 Software, Psychology Software Tools Inc., Sharpsburg, USA). Participants were presented with the CS+, CS-, and neutral pictures from the International Affective Picture System (IAPS) (Lang, Bradley & Cuthbert, 2005). All images were rotated 3° left or right. Image content was irrelevant to the task: participants were instructed to pull or push the joystick in response to rotation direction. Pulling and pushing the joystick respectively gradually increased and decreased image size. Half the participants pushed images rotated left and pulled images rotated right, while the other half received opposite instructions. The CS+, CS- and the neutral pictures were presented 80 times each, 40 times in push- and 40 times in pull-format, resulting in 240 test trials. The latency was recorded between picture onset and completion of a full push or pull response. Literature supports the AAT's validity in measuring approach/avoidance motivational processes (Wiers et al., 2011). Participants

were instructed to perform as quickly and as accurately as possible. Participants completed the AAT before (preconditioning) and after (post conditioning) the experimental conditioning procedure.

The International Index of Erectile Function (IIEF). This is a validated 15-question questionnaire that examines four main domains of male sexual function: erectile function (6 questions, range 0-5), orgasmic function (2 questions, range 0-5), sexual desire (2 questions, range 0-5), and intercourse satisfaction (3 questions, range 0-5). Higher scores indicate better sexual function. Psychometric properties of the IIEF are good (Rosen et al., 1997).

The Female Sexual Function Index (FSFI). Women's sexual functioning was assessed by the FSFI (Rosen et al., 2000; Ter Kuile, Brauer & Laan, 2006), consisting of six subscales: desire (two items; range 1–5), arousal (four items; range 0–5), lubrication (four items; range 0–5), orgasm (three items; range 0–5), satisfaction (three items; range 0–5), and pain (three items; range 0–5). A higher score indicates better sexual functioning. The FSFI has good internal reliability and is able to differentiate between clinical samples and nondysfunctional controls (Wiegel, Meston & Rosen, 2005).

Exit interview. Participants were asked, among others things, about their reactions to the experimental procedure, the use of the genital device, and their evaluation of the genital vibrostimulation. For instance, participants were asked to what extent they liked the vibrostimulation. This could be rated at a 5-point scale ranging from (1) not pleasant at all, to (5) very pleasant. Likewise, participants were asked how sexually aroused they became by the vibration. In addition, they were asked about any prior experience with vibrostimulation. Participants were also asked to rate how successful they were in concentrating and in the deployment of the cognitive strategy on a scale from 1 to 5 (i.e. 1

(trouble keeping concentrated) – 5 (well capable keeping concentrated); and 1 (not successful at all) – 5 (very successful).

9.2.6. Procedure

After participants completed the first session of the AAT, they were instructed that the purpose of the experiment was to measure physiological responses to different pictures and to genital vibrostimulation. Before entering the experimental conditioning session, participants were informed about the vibrostimulation, the colours of the CSs, and the written cues that would appear on screen. Participants were made aware of the contingencies (e.g., only the colour blue or yellow predicted a potential genital vibrostimulation). Participants well-practiced the instructions before commencing the experimental session, and participants were notified that regardless of the written cue, the CS+ always indicated the possibility of receiving genital vibrostimulation. Then the experimenter left the room to allow the participant to place the genital devices privately. Further instructions were given through written instructions on the monitor, and before the experimental procedure started participants were exposed to vibrostimulation for 3 times (periods of 2 s) during which he/she could place the vibrator in the way it was '*most sexually arousing*'. Then a 5-minute resting period followed, during which a neutral film was played and baseline measurements of genital response were collected during the last 2 minutes. Subsequently, the experimental conditioning experiment followed, starting with the preconditioning phase, followed by the acquisition and extinction phases. Directly after this experimental procedure the second session of the AAT was completed. Then participants privately filled in questionnaires (e.g., IIEF, FSFI) and the exit interview questionnaire was administered.

9.2.7. Data Reduction, Scoring and Analysis

Genital data were entered into a computer program (developed by the Technical Support Department of Psychology, University of Amsterdam) that enables offline graphical inspection of the data. A two-pass algorithm for automatic artefact removal was used to analyse the genital data. Artefacts in the channel monitoring VPA and penile circumference are caused by movements of the lower part of the body or by voluntary or involuntary contractions of the pelvic muscles. After artefact removal, mean penile circumference or mean VPA level during the 2-minute resting baseline period was calculated. Genital responses to the CSs were scored in three latency windows: during 4-8, 9-12 and 13-16s following CS onset, respectively FIR (first interval response; during CS presentation), SIR (second interval response; during CS and possible US presentation) and TIR (third interval response; after CS and possible US presentation) (Brom et al., 2014b; Brom et al., 2015a,b). For FIR, SIR and TIR, change scores were calculated for each CS presentation by subtracting mean genital resting baseline from genital measures following CS presentation. Since direct gender comparison of genital responses cannot be made because of the different measures used, genital data for men and women was analysed separately. For genital responses, effects were tested with mixed factor univariate analysis of variance procedures (General Linear Model in SPSS), with Stimulus and Trial as within-subject factors and Condition as between subjects factor. Analyses of subjective measurements and AAT scores were conducted for men and women combined, with Condition and Gender as between subjects factor (General Linear Model in SPSS). The Greenhouse–Geisser correction was applied to adjust for violation of the sphericity assumption in testing repeated measures effects. All phases were analysed separately. The first and second halves of the acquisition phase were also analysed separately. The first extinction trials were analysed separately, since sexual conditioning effects have generally been found to be small and are expected to be strongest on the

first trial directly following the acquisition phase (Brom et al., 2014b, 2015a; Hoffmann, Janssen & Turner, 2004). Also the last extinction trial was analysed separately, since deployment of the emotion regulation strategy is expected to affect not only the magnitude of conditioned responding (extinction trial 1) but also the extinction of conditioned responding (trial 4 of the extinction phase). To correct for outliers, RTs below 200 ms, above 2000 ms and more than 3 standard deviations (SD) above and below the mean were removed for each participant. Error trials were removed. Median RTs were used because they are less sensitive to outliers than means (Cousijn, Goudriaan & Wiers, 2011). Bias scores (median push – pull) were computed for CS+, CS- and the neutral pictures. A positive bias score will be referred to further as an approach bias and a negative bias score as an avoid bias. AAT bias scores were analysed using standard analysis of variance (ANOVA), with Gender and Condition as between-subject factor and Stimulus as within-subject factor with three levels (CS+, CS-, and neutral pictures), and Trial as within-subjects factor with one and two levels (preconditioning and post conditioning). Effect sizes are reported as proportion of partial variance (η_p^2) (Cohen, 1988).

9.3. Results

Men and women differed in age (Men M= 24.26, SD= 6.06; Women M= 28.55, SD= 8.07), $t(90)= -2.79$, $p< .01$, and in prior experience with vibrostimulation (Men M= 1.64, SD= 0.93; Women M= 3.83, SD=1.12), $t(90)= -9.92$, $p< .01$ (see Table 1 for *subject characteristics*). For men, the International Index of Erectile Function Questionnaire (IIEF) Mean score was 35.33 (SD= 5.49), and for women the Mean Female Sexual Function Score was 27.14 (SD = 2.84), indicating sexual functioning within the normal range for both sexes (Rosen et al., 1997, 2000; Ter Kuile, Brauer & Laan, 2006).

Variable:	Men					Women					Men & Women					
	Attend (*n*= 20)		Up-Regulate (*n*= 20)			Attend (*n*= 26)		Up-Regulate (*n*= 27)			Men (N= 40)		Women (N= 53)			Effect size (Cohen's *d*)
	M	SD	M	SD	*p*	M	SD	M	*SD*	*p*	M	SD	M	SD	*p*	
Age (years)	25.00	6.07	23.55	6.13	*.46*	29.04	8.20	28.07	8.07	*.67*	24.26	6.06	28.55	8.07	*<.01*	0.55
Sexual Functioning (IIEF/ FSFI- score)	36.16	5.62	34.55	5.39	*.37*	27.34	2.78	26.96	2.94	*.65*						
Prior Experience Vibrostimulation	1.68	1.06	1.60	0.82	*.78*	3.81	1.30	3.85	0.95	*.89*	1.64	0.93	3.83	1.12	*<.00*	2.12
Pleasantness US	3.16	1.43	3.65	1.04	*.23*	3.50	0.71	3.33	1.04	*.50*	3.41	1.25	3.42	0.89	*.98*	0.01
US Perceived as Sexually Arousing	3.05	1.22	3.35	1.23	*.45*	3.15	0.88	3.15	1.01	*.98*	3.21	1.22	3.15	0.99	*.82*	0.06
Declared Sexual Arousal	2.47	1.43	2.70	0.98	*.57*	2.50	0.76	2.74	1.10	*.36*	2.59	1.21	2.62	.95	*.89*	0.03
Instructions: Able to concentrate	4.11	0.57	3.70	.80	*.08*	3.96	0.45	3.93	.62	*.81*	3.90	0.72	3.94	0.53	*.73*	0.07
Instructions: successful deployment of cognitive strategies	3.95	0.91	3.60	0.88	*.23*	3.92	0.48	3.89	0.64	*.83*	3.77	.90	3.91	0.56	*.41*	0.2

Table 1. Subject characteristics. Descriptive subject variables for men and women, and for each condition.
Notes: IIEF= International Index of Erectile Function FSFI= Female Sexual Function Index. Questions from exit interview. Scales: Prior experience vibrostimulation: 1 (never) – 5 (very often); Pleasantness US: 1 (not pleasant at all) - 5 (very pleasant); US perceived as sexually arousing: 1 (not sexually arousing at all) – 5 (very sexually arousing); Declared sexual arousal: 1 (not sexually aroused) – 5 (very sexually aroused); Instructions: Able to concentrate: 1 (trouble keeping concentrated) – 5 (well capable keeping concentrated); Instructions: successful deployment of cognitive strategies: 1 (not successful at all) – 5 (very successful); * $p < .05$.

9.3.1. Genital Sexual Arousal

Preconditioning phase.

For all latency windows (FIR, SIR and TIR), no difference in penile circumference following presentation of the CS+ and CS- was found, all *p*s > .47. Likewise, for women, no difference in VPA following presentation of the CS+ and CS- was found, all *p*s > .51.

Acquisition phase.

Men. Figure 2 summarizes penile circumference (SIR) to CS+ and CS- across trials for the conditions *Attend* and *Up-Regulate*. A main effect for Stimulus was found on FIR, $F(1, 38)= 12.71$, $p< .01$, $\eta_p^2 =.25$; and SIR, $F(1, 38)= 94.95$, $p< .01$, $\eta_p^2 = .71$, indicating the vibrostimulation resulted in a genital response. In line with earlier studies (Brom et al., 2014b; 2015b) penile circumference was smaller in response to the CS+ and vibrostimulation than in response to the CS-. On TIR no main effect for Stimulus was found, $p= .71$. No interaction effects were found for Stimulus X Condition or Stimulus X Trial X Condition on all time latencies, all *p*s> .19. Additional analysis of only the first 5 trials of the acquisition phase revealed no differences between the two conditions on all time latencies, all *p*s> .16. However, analysis of the last 5 acquisition trials revealed main effects for Condition on all time latencies, FIR $F(1, 38)= 5.24$, $p< .03$, $\eta_p^2 = .12$, SIR $F(1, 38)= 5.45$, $p< .03$, $\eta_p^2 = .13$, TIR $F(1, 38)= 5.64$, $p= .02$, $\eta_p^2 = .13$. This suggests the emotion up-regulatory strategy increased penile responding towards both CSs during the second part of the acquisition phase.

Women. Figure 3 summarizes VPA (SIR) to CS+ and CS- across trials for both conditions separately. The 2 (Stimulus) X 10 (Trial) X 2 (Condition) mixed ANOVA of VPA revealed a significant main effect of Stimulus on FIR, $F(1, 51)= 8.76$, $p< .01$, $\eta_p^2 = .15$, on SIR, $F(1, 50)= 19.42$, $p< .01$, $\eta_p^2 = .28$, and

TIR, $F(1, 50) = 34.24$, $p < .01$, $\eta_p^2 = .41$. No significant Stimulus X Condition, FIR $p = .30$; SIR $p = .65$; TIR $p = .60$, nor Stimulus X Trial X Condition interaction was observed, FIR $p = .38$; SIR $p = .22$; TIR $p = .56$. No main effect of Condition was found, all ps $> .19$. Additional analysis of the first 5 extinction trials of the acquisition phase revealed a significant Stimulus X Trial X Condition effect on SIR, $F(3, 172) = 4.30$, $p < .01$, $\eta_p^2 = .08$. Analyses of the last 5 extinction trials revealed no significant differences between conditions, all ps $>.21$. Meaning in women, the deployment of the emotion up-regulatory strategy increased genital arousal response towards the CS+ and vibrostimulation compared to responses towards the CS- only during the first trials of the acquisition phase.

Extinction phase.

Men. Analysis of the first extinction trial did not reveal a significant main effect of Stimulus, FIR $p = .39$, SIR $p = .29$, TIR $p = .22$, no significant Stimulus X Condition interaction, FIR $p = .14$, SIR $p = .12$, TIR $p = .16$, and no significant main effect of Condition on FIR $p < .07$, SIR $p < .06$, and TIR $p < .06$. The additional 2 (Stimulus) X 2 (Trial; Mean trial 1–4 preconditioning phase and the first extinction trial) Mixed ANOVA revealed no significant Stimulus X Trial X Condition interaction on all time latencies, all ps $> .12$, and no main effect of Condition FIR $p = .08$; SIR $p < .07$; TIR $p = .07$. Analysis of the last extinction trial revealed a significant Stimulus X Condition interaction effect on FIR, $F(1, 38) = 5.99$, $p = .02$, $\eta_p^2 = .14$, and SIR $F(1. 38) = 5.01$, $p = .03$, $\eta_p^2 = .12$, but not on TIR, $p < .06$. As can be seen in Figure 2, men in the *Up-Regulate* condition showed slight increased responding towards the CS- compared to the CS+, whereas men in the *Attend* condition demonstrated increased genital responding towards the CS+ compared to the CS- on the last extinction trial.

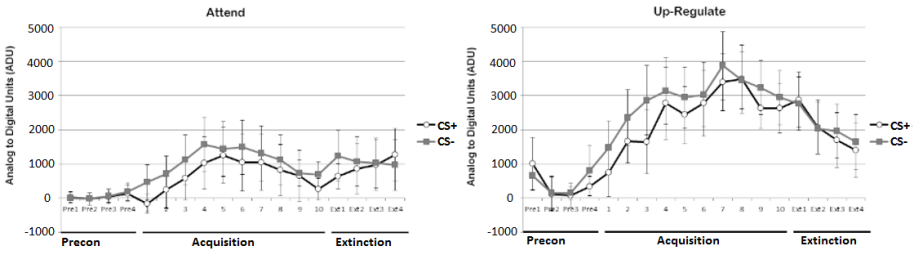

Figure 2. Mean penile circumference change scores (with standard error bars) during the second interval response window (SIR) following the CS+ and CS- during the preconditioning phase, acquisition phase, and extinction phase for the two conditions Attend and Up-Regulate. Note that during the acquisition phase, the response represents responding to the CS+ plus the US. Since not all indium-gallium gauges could be calibrated before data collection, to avoid bias results are calculated with digital output units.

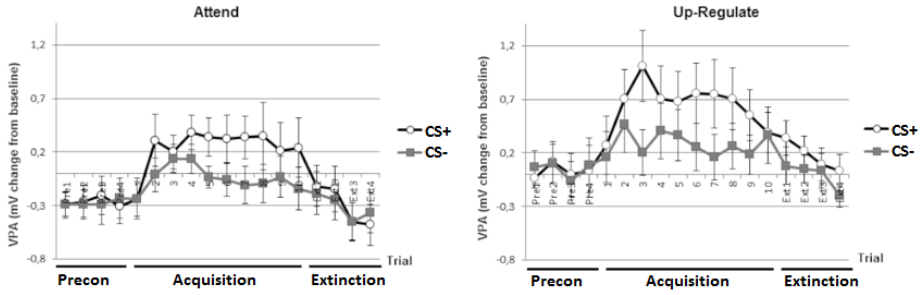

Figure 3. Mean vaginal pulse amplitude (VPA) change scores (with standard error bars) during the second interval response window (SIR) following the CS+ and CS- during the preconditioning phase, acquisition phase, and extinction phase for the two conditions Attend and Up-Regulate. Note that during the acquisition phase, the response represents responding to the CS+ plus the US.

Women. Analysis of the first extinction trial revealed no significant main effect of Stimulus on FIR, p= .26, and TIR, p< .08, but did on SIR, F(1, 52)= 4.86, p= .03, η_p^2 = .09, indicating conditioned responding. No significant Stimulus X Condition interaction was found, FIR p= .93; SIR, p= .20; TIR p= .23, and no main effect of Condition, all ps> .15. The additional 2 (Stimulus) X

2 (Trial; Mean trial 1–4 preconditioning phase and the first extinction trial) Mixed ANOVA revealed no significant differences between conditions on all time latencies, all *p*s> .09.

Analysis of the last extinction trial revealed no main effect of Stimulus on all time latencies, all *p*s> .40, but did reveal a significant Stimulus X Condition interaction effect on SIR, $F(1, 51)= 5.88$, $p<.02$, $\eta_p^2 = .10$. As can be seen in Figure 3, women in the *Up-Regulate* condition showed increased genital response towards the CS+ as compared to the CS- on this last extinction trial, compared to women in the *Attend* condition.

9.3.2. Subjective Measures

Preconditioning phase.

The 2 (Stimulus) X 4 (Trial) X 2 (Condition) X 2 (Gender) mixed ANOVA to verify equal levels of responding to the CSs revealed no difference in responding following presentation of the CS+ and CS- on US expectancy, affective value and sexual arousal value, between conditions and sexes, all *p*s > .15.

Extinction phase.

US expectancy. As can be seen in Figure 4, men and women in both conditions showed a robust differential responding towards CS+ and CS- after the acquisition phase, and both conditions showed a decrease in this differential responding over trials. With other words, men and women expected the US would follow after presentation of the CS+. Analysis of the first extinction trial revealed a significant effect of Stimulus, $F(1, 87)= 233, 55$, $p< .01$, $\eta_p^2 = .73$,

and a significant Stimulus X Gender effect, $F(1, 87) = 32.01$, $p < .01$, $\eta_p^2 = .10$, but no significant Stimulus X Condition interaction, $p = .84$, and no main effect of Gender, $p = .91$. Subsequent analyses for men and women separately, did not reveal differences in conditioned responding between the two conditions, as reflected by non-significant Stimulus X Condition interactions in men, $p = .92$ and women, $p = .84$. The additional 2 (Stimulus) X 2 (Trial; Mean trial 1–4 preconditioning phase and the first extinction trial) Mixed ANOVA revealed no significant Stimulus X Trial X Condition interaction in men, $p = .75$, and women, $p = .77$, and no main effect of Gender, $p = .59$. Analysis of the last extinction trial revealed no significant Stimulus X Condition interactions in men $p = .62$ and women, $p = .51$. No main effects of Condition were found, all ps $> .12$.

Affective value. As can be seen in Figure 5, participants rated the CS+ as more positive compared to the CS- on the first trial of the extinction phase, and this difference in rated subjective affect between CS+ and CS- gradually decreased across trials. Analysis of the first extinction trial revealed a main effect of Stimulus, $F(1, 82) = 37.57$, $p < .01$, $\eta_p^2 = .32$, and an interaction effect of Stimulus X Gender, $F(1, 82) = 7.54$, $p < .01$, $\eta_p^2 = .08$, indicating that men and women differed in conditioned responding after the acquisition phase. Also a main effect of Condition was found, $F(1, 82) = 7.11$, $p < .01$, $\eta_p^2 = .08$. No main effect of Gender was found, $p = .07$.

Analysis of the first extinction trial for men and women separately revealed a significant Stimulus X Condition interaction effect in men, $F(1, 34) = 4.67$, $p < .04$, $\eta_p^2 = .12$, whereas in women it did not, $p = .70$. Meaning, men in the *Up-Regulate* condition demonstrated increased differential responding towards the CS+ and CS- on the first extinction trial as compared with men in

the *Attend* condition. In addition, for men also a main effect for Condition was seen on this first extinction trial, $F(1, 34) = 4.44$, $p = .04$, $\eta_p^2 = .12$. As can be seen in Figure 5, in men the emotion up-regulatory strategy not only resulted in increased differential conditioned responding towards the CS+ and CS- on this first extinction trial, but also resulted in overall higher ratings of affective value towards both CSs.

The additional 2 (Stimulus) X 2 (Trial; Mean trial 1–4 preconditioning phase and the first extinction trial) Mixed ANOVA revealed a significant Stimulus X Trial X Gender interaction, $F(1, 74) = 7.80$, $p < .01$, $\eta_p^2 = .10$, and also a main effect of Gender, $F(1, 80) = 7.17$, $p < .01$, $\eta_p^2 = .08$. This analysis for men and women separately revealed a significant Stimulus X Trial X Condition interaction in men, $F(1, 33) = 4.72$, $p < .04$, $\eta_p^2 = .13$, whereas it did not in women, $p = .94$.

Analysis of the last extinction trial revealed no significant interaction of Stimulus X Condition, $p = .28$, or Stimulus X Gender, $p = .18$, but still revealed a main effect of Stimulus, $F(1, 89) = 5.66$, $p < .01$, $\eta_p^2 = .23$, indicating a difference in rated subjective affect between CS+ and CS- on the last extinction trial with no differences therein between conditions or men and women. However, again a main effect of Condition was found, $F(1, 89) = 4.33$, $p = .04$, $\eta_p^2 = .05$, but no main effect of Gender, $p = .08$. As can be seen in Figure 5, participants in the *Up-Regulate* condition demonstrated overall higher ratings of affective value towards both the CS+ and CS- on the last extinction trial as compared to participants in the *Attend* condition.

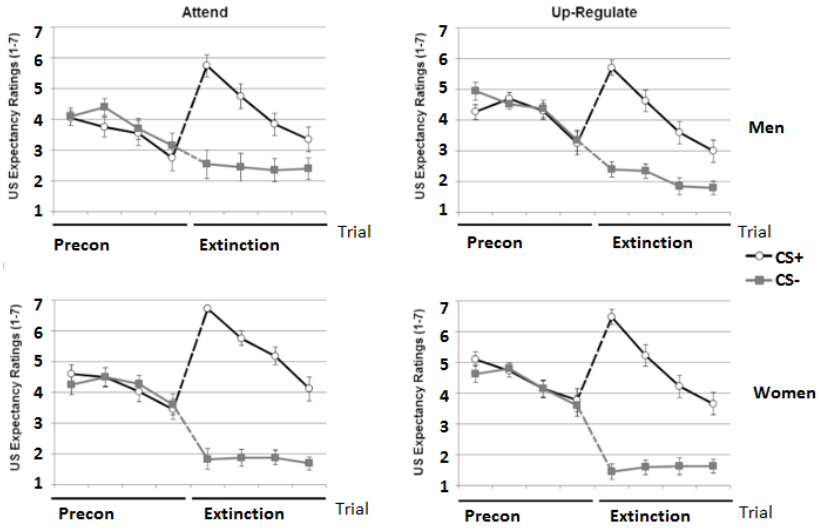

Figure 4. US expectancy ratings (with standard error bars) following the CS+ and CS- during the preconditioning phase and extinction phase for men (top) and women (bottom) in the two conditions Attend (left) and Up-Regulate (right).

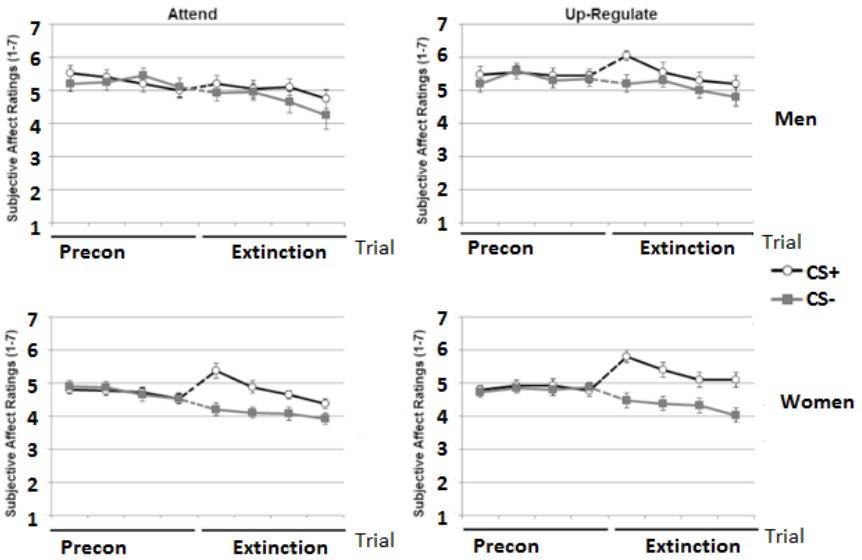

Figure 5. Subjective affect ratings (with standard error bars) following the CS+ and CS- during the preconditioning phase and extinction phase for men (top) and women (bottom) in the two conditions Attend (left) and Up-Regulate (right).

Sexual Arousal Value. Figure 6 shows increased ratings of subjective sexual arousal towards the CS+ on the first trials of the extinction phase in men and women. The 2 (Stimulus) X 2 (Condition) X 2 (Gender) mixed ANOVA of the first extinction trial revealed a significant main effect of Stimulus, $F(1, 85) = 46.67$, $p < .01$, $\eta_p^2 = .35$, and a significant Stimulus X Gender interaction, $F(1, 85) = 4.87$, $p = .03$, $\eta_p^2 = .05$, but no Stimulus X Condition interaction, $p = .75$, and no main effect of Gender, $p = .17$. Further analysis for men and women separately also revealed no significant Stimulus X Condition interactions in both sexes, ps > .81. For men a trend of Condition was seen, $p < .06$.

The additional 2 (Stimulus) X 2 (Trial; Mean trial 1–4 preconditioning phase and the first extinction trial) Mixed ANOVA revealed no Stimulus X Trial X Condition interaction, $p = .51$, and no main effect of Gender, $p = .16$, whereas it did reveal a significant Stimulus X Trial X Gender interaction, $F(1, 80) = 4.48$, $p < .04$, $\eta_p^2 = .05$. Further analyses for men and women separately, revealed no Stimulus X Trial X Condition interaction in men, $p = .78$, and women, $p = .51$. Analysis of the last extinction trial indicated that there was still differential conditioned responding on the last extinction trial, $F(1, 88) = 23.76$, $p < .01$, $\eta_p^2 = .21$. The analysis did not reveal significant Stimulus X Condition and Stimulus X Gender interactions, all ps > .11. Also no main effect of Condition, $p = .08$ or Gender, $p = .07$ was found.

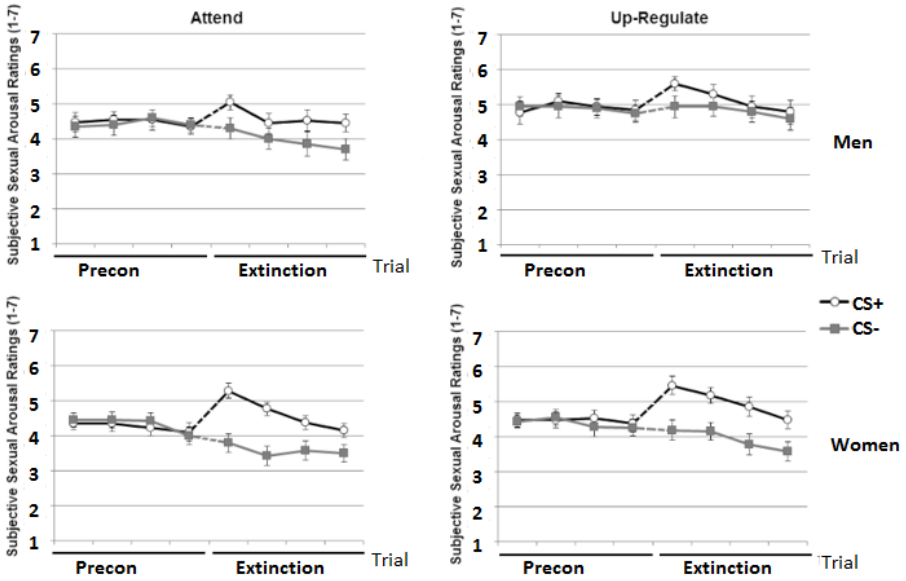

Figure 6. Ratings (with standard error bars) of sexual arousal value towards the CS+ and CS- during the preconditioning phase and extinction phase for men (top) and women (bottom) in the two conditions Attend (left) and Up-Regulate (right).

9.3.3. Approach and Avoidance Tendencies

The preconditioning AAT bias scores were analysed with a mixed ANOVA with Gender and Condition as between-subject factor and Stimulus as within-subject factor with three levels (CS+, CS-, and neutral pictures). In line with the expectations, no interaction effect was found for Stimulus and Condition, p= .98, and men and women also did not seem to behave differently in approach and avoidance tendencies towards the stimuli before the conditioning procedure, as reflected by the non-significant Stimulus X Gender interaction, p= .85.

The mixed ANOVA with Gender and Condition as between-subject factor, and Stimulus as within-subject factor with three levels (CS+, CS-, and neutral pictures), and Trial as within-subjects factor with two levels

(preconditioning and post conditioning), of the AAT bias scores, revealed a Stimulus X Trial X Gender, $F(1, 145) = 24.08$, $p < .01$, $\eta_p^2 = .22$, and Gender X Condition interaction effect, $F(1, 88) = 5.22$, $p < .03$, $\eta_p^2 = .06$. No Stimulus X Trial X Condition effect was observed, $p = .47$. Analysis for men and women separately, revealed no significant effects of Stimulus or Stimulus X Trial for men, all ps $> .07$, whereas for women a significant Stimulus X Trial interaction, $F(2, 82) = 61.74$, $p < .01$, $\eta_p^2 = .54$, and significant main effect of Stimulus, $F(1, 81) = 64.48$, $p < .01$, $\eta_p^2 = .55$, was found. In men only a main effect of Condition was found, $F(1, 37) = 4.32$, $p < .05$, $\eta_p^2 = .10$. As can be seen in Figure 7, men in the *Up-Regulate* condition had overall higher bias scores towards all stimuli, both preconditioning and post conditioning.

Analysis of only the post conditioning AAT scores demonstrated a significant main effect of Stimulus, $F(1, 132) = 40.81$, $p < .01$, $\eta_p^2 = .31$, and interactions of Stimulus X Gender, $F(1, 132) = 43.32$, $p < .01$, $\eta_p^2 = .32$, and of Gender X Condition, $F(1, 89) = 5.27$, $p = .02$, $\eta_p^2 = .06$. No significant Stimulus X Condition interaction was found, $p = .20$. Analysis of post conditioning bias scores for men and women separately, demonstrated a main effect of Stimulus in women, $F(1, 65) = 87.14$, $p < .01$, $\eta_p^2 = .63$, indicating conditioned responding, whereas in men it did not, $p = .62$. As can be seen in Figure 7, in line with the expectations, women in both conditions demonstrated a conditioned approach bias towards the CS+ compared to the other stimuli (i.e. CS- and neutral pictures). However, no Stimulus X Condition interaction effects were found in both sexes, men $p = .75$, women $p = .40$, indicating the

emotion regulatory strategy did not affect conditioned differential behavioural approach and avoidance tendencies towards the CS+, CS- and neutral stimuli.

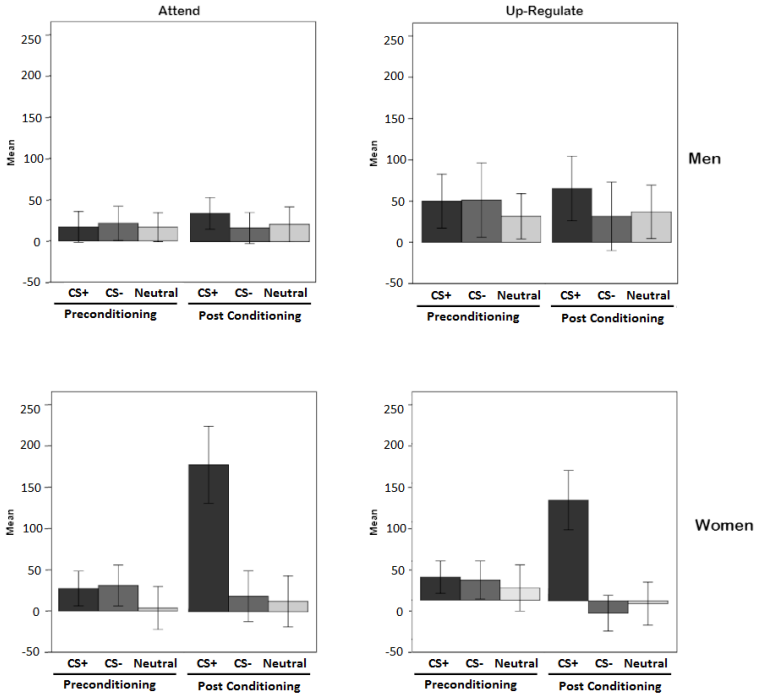

Figure 7. Approach Avoidance Task (AAT) bias scores for CS+, CS-, and neutral images in men (above) and women in the Attend and Up-Regulate condition (ms with standard error bars), preconditioning and post conditioning. A positive score indicates faster reaction times on approach (pull) trials compared to avoid (push) trials.

9.4. Conclusions

In the current study, genital, subjective and behavioural correlates of the interaction of emotion up-regulation with sexual conditioning were investigated. Consistent with findings from previous studies, conditioning

effects were observed (Both et al., 2008; Brom et al., 2014a,b, 2015b) and in line with findings from a previous emotion regulation study on conditioned sexual response (Brom et al., 2015b), sexual arousal could be modulated in line with participants' regulatory goals. In men, CRs were found on measures of subjective affect, sexual arousal value, and US expectancy, and no extinction thereof on the last extinction trial. However, no evidence was found for conditioned genital response or conditioned approach tendencies towards the CS+. In women, CRs were seen on all measures, and like in men, on all subjective measures no complete extinction of conditioned responding was seen. Thus, in both men and women, a picture of the opposite sex that was repeatedly followed by genital stimulation was evaluated as more positive and as more sexually arousing, and in women, this picture also elicited conditioned genital response and approach tendencies.

Second, regarding the sexual arousal emotion up-regulatory strategy, in men and women, the deployment of such a strategy did not increase genital arousal responses in response to the CS+ (and vibrostimulation) compared to the CS-, but the cognitive up-regulatory strategy increased overall genital responding towards both CSs in the acquisition phase. However, the sexual arousal up-regulatory strategy did not seem to affect the magnitude of conditioned responding in men and women on the first extinction trial. Nevertheless, the deployment of the cognitive up-regulatory strategy seemed to result in enhanced resistance to extinction of conditioned genital responding in women, since only women in the *Up-Regulate* condition still showed conditioned genital response on the last extinction trial, whereas women in the *Attend* condition did not. With respect to the subjective measures, in men, the emotion up-regulatory strategy not only resulted in increased conditioned positive affect on the first extinction trial, but also resulted in overall higher ratings of positive value towards both CSs. These results indicate that in men, affective value can

be up-regulated by cognitive strategies. In contrast, in women, the cognitive up-regulation strategies did not seem to have an effect on subjective affective value. On measures of sexual arousal value and US expectancy the emotion up-regulatory strategy did not seem to affect conditioned responding or extinction thereof, in both sexes. And lastly, the emotion up-regulation strategy did not result in increased approach tendencies towards the CS+ in men and women. In line with earlier studies (Brom et al., 2015b) the cognitive regulatory strategy mainly operated on physiological measures of sexual response and valence, leaving the more cognitive aspects (US expectancy) of conditioning intact (Boddez et al., 2013). And although, based on the literature, effects on autonomic physiological responses (i.e. expectancy learning) were not expected (Baeyens et al., 1992; Blechert et al., 2015; De Houwer, Thomas & Baeyens, 2001) results from the present study and a former study (Brom et al., 2015b) demonstrate that cognitive regulatory strategies seem to be able to affect extinction of conditioned physiological responding.

Although it is speculated that women may use less effective cognitive strategies compared to men (Brom et al., 2015b; Whittle et al., 2011), given the problems in comparing genital responses of men and women directly, and possible differences between sexes with regard to responses to specific types of stimulus materials, and the actual deployed ER technique it is far too early to infer that women indeed are less efficient in the up-regulation of positive (sexual) emotions than men. Some ER strategies are likely less costly to implement (e.g., distraction or increasing attentional focus), which may offer advantages even when these strategies are less effective long-term (e.g., compared to reappraisal) (Moyal, Henik & Anholt, 2013). Importantly, Moholy and colleagues (Moholy et al., 2015) demonstrated that the level of sexual desire was shown a primary predictor of sexual regulation. Since it is widely accepted that men and women differ in strength of sex drive (Baumeister et al., 2001),

this difference in level of sexual desire and sex drive may account for the found differences between men and women in research on the regulation of sexual arousal.

Second, it is important to keep in mind that the effect of the emotional up-regulatory strategy in the present study is relative to the other (*Attend*) strategy with which it is compared and does therefore not reflect the complexities of the emotion regulation repertoire (Aldao, 2013). Future studies should therefore investigate if the found gender differences are also seen making use of multiple cognitive up-regulatory strategies, including more response-focused strategies (Gross & Thompson, 2007). However, in a study on the regulation of sexual arousal by means of attentional focus in healthy sexually functional men and women, Both, Laan and Everaerd (2011) found interesting gender differences. When taking a participant and emotion-oriented ('hot') focus rather than a spectator and stimulus-oriented ('cool') focus while viewing erotic stimuli, participants were able to enhance feelings of sexual arousal. Intriguingly, women reported stronger absorption (i.e. the extent to which the participant experienced him or herself as a participant in the sexual activity shown in the film) in the cool attentional focus condition than in the no-instruction control condition, whereas men, as expected, reported lower absorption levels in the cool attentional focus condition than in the no-instruction control condition. A possible more pronounced difficulty in emotion regulation in women while processing sexual (conditioned) stimuli (Both et al., 2011; Brom et al., 2015b), may be the result of anatomical differences between men and women (Laan & Everaerd, 1995). Bodily responses and changes therein are an apparent aspect of emotional response (Damasio, 2003). The association between genital and subjective sexual arousal is generally lower for women than for men (Chivers et al., 2004). Men are likely to have more (visual and tactile) cues they can use to detect genital response

than women do (Sakheim et al., 1984). Nevertheless, in women the emotion up-regulatory strategy did result in enhanced resistance to extinction of conditioned genital response, and in men, the only prominent effect of the up-regulatory strategy was seen on affective value and not on conditioned genital sexual response. Maybe the fact that only healthy sexually functioning subjects participated in this study can contribute for this. Healthy young men likely have less experience with the up-regulation of sexual arousal compared to down-regulation of sexual arousal, since the expression of sexuality is not always accepted or appreciated in daily life, and instances of needing to increase sexual arousal are likely less common in healthy participants. The majority of the empirical investigations on emotion regulation (Aldao, 2013), including the present study, have examined processes in healthy individuals, and only little attention has been devoted to how those processes might differ as a function of variability in psychopathology status. As it is suggested that personality facets and dispositional and state-level psychological processes influence emotion regulatory processes (Aldao, 2013), an important venue for future research is the tailoring of the emotion regulation strategies to clinical samples, such as individuals with low sexual arousal and desire.

In the present study no ratings of US expectancy, affective value and sexual arousal value were collected during the acquisition phase. Since this information is essential in clarifying which type of measures of sexual response cognitive up-regulatory strategies are effective, future studies on the effectiveness of cognitive strategies on sexual arousal should also collect those subjective measures during acquisition. Furthermore, another limitation of the present study is the absence of a between-subjects (unpaired) control group. Without such a control group it is difficult to determine whether and which type of learning has occurred. At present it is unclear if the observed differential response towards the CS+ and CS− was due to conditioning or to

pseudo-conditioning. The possibility of sensitization of sexual arousal would translate into increased genital responses across trials, and not in differential responding towards the CS+ and CS− per se (Domjan, 2010; Hoffmann et al., 2014). Therefore, making use of such a control group in future research is desirable. Additionally, in the present study the genital arousal results during acquisition and extinction could be influenced by carry-over effects, also resulting into overall increased genital responses across trials. Future studies should consider implementing a return-to-baseline design. Although the random presentation of CS+ (plus vibrostimulation) and CS- in the acquisition phase can only control to a certain extent for potential carry-over effects, these possible effects are equally expected in the *Attend* and *Up-Regulate* condition. Therefore, any effects of the experimental conditions may be attributed to the experimental manipulation (i.e. deployment of the Up-Regulatory strategy) rather than carry-over effects. Furthermore, in the present study, vaginal photoplethysmography and penile circumference was used as indicator of physiological sexual arousal. Vaginal and penile engorgement, however, is only one of many co-occurring processes during the sexual arousal response. Ideally, future studies should incorporate other methodology, such as thermal imaging or neuroimaging to allow for better investigation of small sexual CRs and comparison between men and women. Next, the present study did not control or quantify the used regulation strategies. However, despite these limitations in design, differences between conditions in differential responding towards the CS+ and CS- could be observed, suggesting that making use of this less stringent control design (i.e. only the CS- as control measure) still enabled to test for effects of the experimental conditions.

To conclude, the present results suggest that in the treatment of problematic low sexual arousal, cognitive up-regulatory strategies of sexual arousal may be applied during initial conditioning stages in CBT in men and

women. Results from the acquisition phase point to the utility of up-regulatory training for enhancing genital sexual arousal during the learning of new associations of sexually rewarding experiences and stimuli. In addition, the cognitive strategy also substantially enhanced resistance to extinction of conditioned genital response in women, and increased conditioned positive valence in men, making it a promising add-on tool during therapeutic exercises in order to (re)create and enhance sexually pleasurable experiences. However, future studies should assess the clinical efficacy of cognitive up- and down-regulatory strategies by including clinical samples, such as individuals with low sexual arousal and desire. Additionally, future studies should also investigate the (clinical) effectiveness of other strategies such as mindfulness (Goldin & Gross, 2010; Kumar, Feldman & Hayes, 2008), or hot/cool focus on conditioned sexual response.

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Chapter 10

The Influence of Acute-Stress on the Down-Regulation of Sexual Arousal

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Abstract

Although it is hypothesized that dysregulated emotion regulation (ER) and vulnerability to stress play an important role in problematic sexual reward seeking behaviour, little is known about their interaction and neural correlates. In the present study, we investigated the neural effects of an emotion down-regulatory strategy during the processing of sexual stimuli, and the influence of acute stress thereon. It was hypothesized that activation within reward related structures (i.e. NAc, amygdala) decreases consistent with down-regulation, while activation in prefrontal control areas would increase, and that acute stress (induced by the Trier Social Stress Test; TSST) would affect the ability to down-regulate sexual arousal. Acute stress was thought to increase NAc and amygdala activation, and decrease activation in dorsolateral frontal control areas, while increasing activation in ventral frontal areas. Participants were randomly assigned to a stress (n=20), or control condition (n=18), and had to increase (´Sex-Up´), decrease (´Sex-Down´) or maintain ('Sex-Equal') their sexual arousal response evoked by sexual explicit pictures inside a MRI-scanner. BOLD responses to sexual pictures were compared to neutral pictures on viewing trials ('Neutral-Equal'), and ratings of the ability to regulate sexual arousal were analyzed. Across both conditions, down-regulation of sexual arousal activated prefrontal regions. Crucially, compared to the control condition, induced stress resulted in increased activation in the right amygdala, right dorsal anterior cingulate cortex (ACC) and right inferior frontal gyrus pars triangularis (IFG) in Sex-Down vs Sex-Equal trials. Results suggest that acute stress may markedly impair the cognitive down-regulation of sexual arousal and highlights critical limitations of this technique to control sexual arousal under stress.

10.1. Introduction

Stress is widely acknowledged as a risk factor for the development of a wide range of disorders, including disorders of excessive appetite such as substance use disorders, gambling addiction, or sexual addiction (Adam & Epel, 2007; Uhart & Wand, 2009; Reid et al., 2008, 2012). Research has shown that individuals that manifest symptoms of hypersexual behaviour are more likely to experience vulnerability to stress and deficits in emotion regulation (ER) (Reid, 2014). Although it is hypothesized that ER and vulnerability to stress play an important role in sexual reward seeking behavior, much remains unknown about their interaction and neural correlate. Given the pervasive nature of stress in daily life, it is critical to understand how acute stress may influence this ability to modify sexual arousal.

Sexual arousal can be seen as an evolutionary preserved emotion (Everaerd, 1989; Frijda & Sundararajan, 2007; Janssen, Everaerd, Spiering, & Janssen, 2000). Sexual arousal is characterized by specific bodily reactions, like enhanced genital blood flow, by preparation of behavioural action, and by the experience of feelings of lust, excitement, and sexual desire, and can eventually result in overt sexual behaviour such as approach and consumption (Both et al., 2005; Dekker & Everaerd, 1989; Lang, 1971). Disturbances in the regulation of sexual arousal might be a key factor in the genesis of disorders in sexual motivation, such as hypersexuality, which may involve a chronic inability to suppress sexual arousal. ER is any process by which an individual modulates the intensity and direction of emotional response (Gross, 2002; Gross & Thomspon, 2007). Emotional down-regulation refers to the process of inhibiting the emotional response, or the intensity of the emotional response, regardless of its valence. The ability to regulate emotions when stressed is considered essential for mental health and deficit of such capacity confers risk

towards psychopathology (Gross, 2002; Heatherton & Wagner, 2011; John & Gross, 2004).

On a neural level, successful emotion down-regulation is thought to reflect the inhibition of subcortical brain areas related to emotional response, such as the amygdala, mediated by regions of the prefrontal cortex (PFC) that appear to act as control systems that implement the regulatory strategy (Frank et al., 2014, Ochsner et al., 2012; Ochsner & Gross, 2005). The technique 'reappraisal' (i.e. cognitive change, yielding an altered interpretation of an emotional stimulus or situation) has been proposed to be an effective ER strategy to down-regulate emotions because its influence begins at an early stage of emotion generation, before emotional responses have fully unfolded (Ochsner & Gross 2005; Richards & Gross, 2000). Research on the neural correlates of reappraisal of negative emotions has demonstrated that activation of the amygdala can be reduced during reappraisal (Ochsner et al., 2002; Phelps, 2006). This reduction in amygdala activation is negatively related to activity in a neural network including the anterior cingulate cortex (ACC), and frontal control areas, such as the orbitofrontal cortex (OFC), the inferior frontal gyrus (IFG) the middle frontal gyrus (MFG), and the parietal cortex (Banks et al., 2007; Etkin, Egner & Kalisch, 2011; Grecucci et al., 2013; Hamann, 2007; Kim & Hamann, 2007; Kim & Ochsner et al., 2002, 2004; Shenhav, Botvinick & Cohen, 2013). Moreover, it is important to keep in mind that the principles underlying ER also form the basis of cognitive-behavioral therapy (CBT), an intervention widely used in the clinic to treat individuals with hypersexuality (Birchard, 2015). This implies that the success of this technique for controlling maladaptive emotional responses relies on the availability of cognitive resources and intact executive function (Heatherton & Wagner, 2011; Raio, 2013). Despite the presumed importance, compared to the substantial amount of research on down-regulation of negative emotions, research assessing the dynamic interactions between regions within the subcortical

reward structures and the frontal circuit during the active cognitive control of sexual arousal is extremely scarce. Nevertheless, there is evidence for the involvement of prefrontal areas in the regulation of sexual arousal. For instance, using functional near-infrared spectroscopy, Leon-Carrion et al. (2007) demonstrated that even after sexual stimuli presentation ceased, dorsolateral PFC activation continued, supporting the involvement of frontal areas in the regulation of sexual arousal. Additionally, Beauregard et al. (2001) found that men, when instructed not to suppress becoming sexually aroused by sexual stimuli, demonstrated significantly enhanced activation in the right amygdala, right anterior temporal pole, and hypothalamus, whereas men that were instructed to suppress sexual arousal demonstrated activation in the right superior frontal gyrus and in the right ACC. In sum, successful ER appears to involve a balance between subcortical brain regions related to emotional response (e.g., amygdala) and prefrontal regions associated with cognitive control (Heatherton & Wagner, 2011).

A vast number of investigations using functional MRI have consistently shown that visual sexual stimuli evoke activations in the brain's reward system, such as the NAc and amygdala (Childress et al., 2008; Georgiadis & Kringelbach, 2012; Gillath & Canterberry, 2012; Hamann et al., 2004; Oei et al., 2012a; Rupp & Wallen, 2008; Stoléru et al., 2012). NAc activation is modulated by dopamine (DA) signaling (Richard et al., 2012), with higher activations in response to sexual reward cues when DA activity is increased, and lower activations when DA activity is decreased (Oei et al., 2012a). Interestingly, stress might increase sensitivity to potentially rewarding stimuli through its effects on DA signaling in the NAc (Cabib & Puglisi-Allegra, 2012; Oei et al., 2014). Additionally, a growing body of work has revealed that exposure to acute stress has deleterious effects on the successful execution of higher cognitive processes, including ER (Arnsten, 2009; Heatherton & Wagner, 2011; Kogler, Gur & Derntl, 2015; Raio et al., 2013). It

is thought that neuroendocrine responses to acute stress exposure impacts the functional integrity of the PFC (Arnsten, 2009; Arnsten, Wang & Paspalas, 2012), which is, as has become clear, crucial in successful cognitive ER (Beauregard, 2007; Hartley & Phelps, 2010; Ochsner et al., 2004; Ochsner & Gross, 2005; Ochsner, Silvers & Buhle, 2012; Levesque et al., 2003; Phan et al., 2005; Urry et al., 2006). Moreover, stress seems to activate ventral 'affective' brain areas, while deactivating dorsolateral 'executive' prefrontal areas during emotional inhibition (Oei et al., 2012b). Importantly, since impulse control is at the core of self-regulation (Heatherton & Wagner, 2011), increased neural activity in subcortical reward structures such as the amygdala and NAc as a result of induced stress (Oei et al., 2014), may further impede successful top-down control recruitment. This suggests an important paradox: top-down control may be compromised in regulating sexual arousal at times when such cognitive control is needed most, since acute stress can activate the brain reward system (Oei et al., 2014), resulting in increased bottom-up subcortical responses.

The aim of the present study is to investigate the influence of acute stress on the cognitive regulation of sexual arousal in an experimental design, in which young healthy men were randomly allocated to an experimental or control condition. The experimental condition underwent a stress procedure before they had to increase (´Up´), decrease (´Down´) or maintain ('Equal') their sexual arousal response evoked by sexual explicit pictures inside a MRI-scanner. We aimed at examining functional activations in regions associated with sexual reward, such as the NAc (Stoléru et al., 2012; Oei et al., 2012a), and amygdala (Hamann et al., 2004), and frontal regions involved in emotional response and implementing cognitive ER strategies, such as the IFG pars triangularis (Aron et al., 2007; Aron, Robbins & Poldrack, 2014; Hampshire et al., 2010; Shamay-Tsoory, Aharon-Peretz & Perry, 2009), the MFG (Etkin, Egner & Kalisch, 2011), and the dorsal ACC (Mohanty et al., 2007; Shenhav,

Botvinick & Cohen, 2013), during down-regulation of sexual arousal. It was predicted that activation within the NAc and amygdala would decrease consistent with emotional down-regulation, while activation in the IFG pars triangularis and MFG would increase (Amaral & Price, 1984; Baumann & Turpin, 2010; Ghashghaei et al., 2007; Ochsner et al., 2004; Phelps et al., 2004). Crucially, it was hypothesized that acute stress would affect the ability to down-regulate sexual arousal by increasing activations in ventral 'affective' areas, such as the amygdala, and decreasing activation in dorsolateral frontal control areas such as the MFG, while demonstrating increased activation in ventral 'affective' frontal areas, such as the IFG pars triangularis.

10.2. Methods and materials

Participants

A total of 40 men from the general population were recruited by means of advertisements. Eligibility criteria were: no current (or history of) psychiatric problems as determined by the Amsterdam Biographical interview (ABV; Wilde 1963) and the MINI International Neuropsychiatric Interview (MINI; Sheehan et al, 1998), a heterosexual orientation, no medical illness (or medical history) or use of medication, including over the counter hay fever medication; no current or recent use (less than 12 weeks before participation) of psycho-pharmacological medication or psychotropic drugs; alcohol usage below 20 units per week. Participants were randomly assigned to the stress or control condition in an experimental design. The study was approved by the medical ethical committee of the Leiden University Medical Center and written informed consent was given by all participants.

Stress-induction & physiological assessments

The stress induction procedure has been described in detail elsewhere (Oei et al., 2014). In brief, the Trier Social Stress Test (TSST; Kirschbaum et al., 1993)

consists of a 10-min period in anticipation of a 5-min free speech, and a 5-min arithmetic task (counting backwards from 1033 to zero, in steps of 13) in front of a selection committee and a camera. In contrast, in the control condition, participants have to prepare and conduct a speech without audience about a book or movie. Thereafter, they have 5 min to count backwards from 50 to zero at their own pace (Het et al., 2009). Salivary cortisol was assessed, using salivettes (Sarstedt, Germany). Systolic blood pressure (SBP, mmHg), diastolic blood pressure (DBP, mmHg), and heart rate (HR, bpm) were recorded using an automatic wrist blood pressure monitor (OMRON, R5-I) (see Oei et al., 2014).

Materials

An ER task was presented during fMRI scanning, and consisted of ten neutral images and 40 erotic pictures that were selected from the International Affective Picture Set (IAPS; Lang et al., 2008). Additional erotic images were selected from picture sets previously used in sexology research and depicted (partly) naked humans in a heterosexual erotic context (Both et al., 2004; Brom et al., 2015a). Each trial began with a brief written cue on screen (2 s), 'equal', 'increase' or 'decrease', that was immediately followed by a neutral or sexual picture (duration = 8 s), see Figure 1. The assignment of the images to instructions was randomized and counterbalanced across subjects, with the restriction that written instructions were equally matched to stimulus category (Neutral pictures: 10 trials do not modify, i.e. 'Neutral-Equal'; Erotic pictures: 10 trials do not modify, i.e. 'Sex-Equal'; 15 trials increase, i.e. 'Sex-Up'; 15 trials decrease, i.e. 'Sex-Down'). After each trial participants were asked to indicate whether they felt their emotions were successful modulated according to the strategy when asked to apply, up- or down-regulation or not to modify their emotion on each of these trials, via button response on a 4-point scale (1 not successful – 4 very successful) (8s). There was an inter-trial interval showing a

gray fixation dot with a random duration between 2s and 6s for jitter. Stimuli were presented in an 800 X 600 pixel resolution, back-projected on a screen located at the end of the scanner bore via an LCD projector located outside the scanner room. Subjects viewed stimuli on a screen through a mirror located on the head coil. Stimulus software (E-prime 2, Psychology Software Tools, Inc.) was used for stimulus presentation. Refresh rate of both the task PC monitor and projector was 60 Hz.

Instructions: Three task conditions were randomly presented. In the view condition (i.e. Sex-Equal and Neutral-Equal), when the instruction '*equal*' (i.e. do not modify) was presented, participants attended the content of the picture but did not manipulate the emotional response to it. When participants received the instruction '*increase'* prior to a picture, they were instructed to increase or up-regulate any experienced or felt sexual response and arousal the picture might elicit. More specifically, they were instructed to identify as much as possible with the male actor or to imagine that they themselves were engaged in the sexual activities depicted in the picture (i.e. Sex-Up). When participants received the instruction '*decrease'*, they were instructed to reappraise and down-regulate the emotional value of the images, so that the sexual impact was lessened (i.e. Sex-Down). Participants were instructed to generate a more distant interpretation of the scene depicted in the picture. More specifically, when presented with the instruction *'decrease'*, they were instructed to remind themselves that they were simply watching a picture depicting actors playing a role. A comprehensive prescanning training procedure was used to assure that participants understood the cue-task associations and the reappraisal strategy.

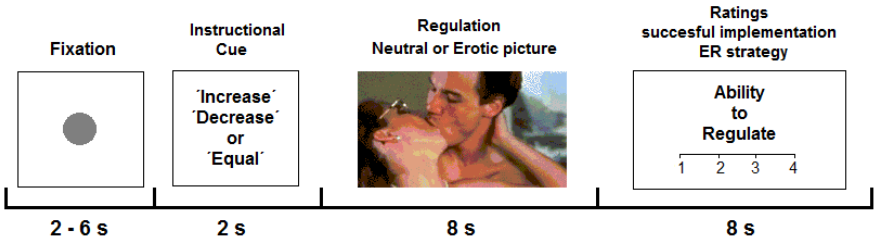

Figure 1. Experimental design for a single trial. The experiment consisted of 50 trials (10 neutral pictures and 40 erotic pictures). There were 10 trials each of do not modify (i.e. *equal*) emotional response in response to neutral pictures ('Neutral-Equal') and erotic pictures ('Sex-Equal'), and 15 trials of *increase* sexual arousal in response to erotic pictures ('Sex-Up'), and 15 trials of *decrease* sexual arousal in response to erotic pictures ('Sex-Down').

Scan protocol

Imaging was carried out on a 3 T Philips Achieva MRI scanner (Philips, Best, The Netherlands), using a 32-channel SENSE head coil. A standard T1-weighted structural volume and a high resolution gradient echo EPI scan were acquired for registration purposes. For fMRI during the emotion regulation task, T2*-weighted gradient echo planar images (EPI) sensitive to BOLD contrast were obtained in the axial direction (echo time 30 ms, flip angle 80º, isotropic voxels of 2.75 mm, 0.25 mm slice gap, 38 slices, repetition time 2.2 s).

Procedure

All participants arrived in the morning at either 08:00 h, or 09:15 h. The arrival time of the participants was balanced between and within stress and control condition, to keep morning cortisol levels as even as possible over groups. After informed consent was given, and the participants changed into the obligatory hospital clothing, the TSST protocol started with instructions (i.e., to prepare a presentation). After 10 min preparation time, participants were brought to a room, in which the committee was seated, and the TSST protocol was continued for 10 min (Kirschbaum et al., 1993; see Oei et al., 2014 for

detailed TSST-procedure). After the TSST, the participants were brought to the scanner, in which the emotion regulation task was delivered, approximately 20 min after the end of the TSST. The emotion regulation task was preceded by two other scanner tasks and structural scans. Saliva was sampled at four times: immediately before TSST instructions ("baseline") and after the preparation phase of the TSST ("pre-speech"), at the end of the TSST, just before entering the scanner ("post-TSST"), and immediately after the scan procedure ("post-scan"). Blood pressure, heart rate and subjective stress were sampled at the same time points. After scanning, participants filled out questionnaires, and completed an exit interview. Thereafter, a debriefing regarding the TSST followed. Participants were thanked and received financial compensation for their participation.

Data processing and analysis

Physiological data and subjective ratings of stress Cortisol samples, blood pressure, heart rate and subjective stress were analyzed with repeated measures (RM) ANOVAs, and independent *t*-tests performed at baseline, after stress, and after scanning. Greenhouse- Geisser correction was applied when appropriate.

FMRI data processing was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB's Software Library, www.fmrib.ox.ac.uk/fsl). The following pre-statistics processing was applied; motion correction; non-brain removal; spatial smoothing using a Gaussian kernel of FWHM 5 mm; grand-mean intensity normalization of the entire 4D dataset by a single multiplicative factor; high pass temporal filtering (Gaussian-weighted least-squares straight line fitting, with s = 25.0s). Time-series statistical analysis was carried out with local autocorrelation correction. FMRI EPI data were registered to the high resolution EPI scan of each participant, which was registered to the individual T1-weighted structural scan, which was registered to the MNI-152 standard space template. Six explanatory variables (EV) were

included in the general linear model: the 4 target categories, neutral - no modified emotional response (Neutral-Equal), sexual - no modified emotional response (Sex-Equal), sexual - increased emotional response (Sex-Up), sexual - decreased emotional response (Sex-Down), and 'Rating' and 'Instruction', each time-locked to the target onset. Each EV was convolved with a double gamma hemodynamic response function. Contrasts of interest were Sex-Down versus Sex-Equal and Sex-Down versus Neutral-Equal. For whole brain analysis, the images of contrasts of parameter estimates and corresponding variances were fed into a higher-level mixed effects analysis, carried out using FLAME (FMRIB's Local Analysis of Mixed Effects) with automatic outlier detection. To determine main task effects, irrespective of condition assignment, a one-sample *t*-test was done. Herein, whole brain *Z* (Gaussianised *T*) statistic images were thresholded by an initial cluster-forming threshold of $Z > 2.3$ and a (corrected) cluster significance threshold of $p = .05$.

Subsequently, to investigate the effects of Condition (Stress vs Control) we focused on ROIs for which a role in sexual arousal, emotion regulation and inhibitory control in relation to urges has been demonstrated before, and which were a priori hypothesized to be affected by stress, i.e. the NAc (Stoléru et al., 2012; Oei et al., 2014), amygdala (Hamann et al., 2004; Oei et al., 2012b), dorsal ACC (Shenhav, Botvinick & Cohen, 2013), the MFG (Ochsner et al., 2012) the right IFG pars triangularis (Aron et al., 2007; Aron, Robbins & Poldrack, 2014; Hampshire et al., 2010), and OFC (Banks et al., 2007). Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z>2.3$ and a (corrected) cluster significance threshold of $p = .05$ (Worsley 2001). The binarized images of the NAc and amygdala from the Harvard-Oxford Subcortical Probability Atlas were used as anatomical masks, set at a probability of 50%. Likewise, the binarized images of the OFC, MFG and IFG pars triangularis from the Harvard-Oxford Cortical Probability Atlas were used as anatomical masks, also set at a probability of 50%. For the dorsal ACC, an

atlas-based mask was made, removing the subgenual part of the ACC at MNI coordinate *y*= 32 (McCormick et al, 2006). In case the ROI analyses yielded significant differences between the two conditions, correlation analyses were performed between Featquery zstats of the a-priori defined ROIs and subjective ratings of successful implementation of the cognitive regulatory strategies. Correlation analyses were performed using IBM SPSS statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA).

10.3 Results

One participant was discarded in the analysis because of acute personal stress that he reported at arrival (initial stress was accompanied by extreme baseline salivary cortisol levels >50 nmol/L). One participant was excluded due to significant movement in the scanner. Both were from the control condition. The final sample thus consisted of 38 participants; 18 control participants (mean age 21.50 ± 2.90 years) and 20 participants who were exposed to psychosocial stress (mean age 22.42 ± 3.25 years). The stress and control condition did not differ in terms of age, BMI, psychoneuroticism as assessed with the Symptom Checklist-90, the sensitivity of two motivational systems (i.e. the appetitive and aversive system) as assessed with the Behavioral Inhibition Behavioral Activation Scales, BIS/BAS (Carver & White, 1994), and baseline heart rate, blood pressure or cortisol (see Oei et al., 2014). Although participants in both conditions scored within the normal range on the Sexual Inhibition (SIS) & Sexual Excitation Scale (SES) (Janssen et al., 2002a,b), the conditions differed in individual propensities to become sexually aroused and to be sexually inhibited (SIS1 [threat of performance failure], control condition: 36.61 ± 2.55; stress condition 36.53 ± 3.20, p= .93; SIS2 [threat of negative consequences], control condition 27.89 ± 3.16; stress condition 25.42 ± 2.81, $t(35)$= 2.51, p< .02; SES, control condition 50.94 ± 4.43; stress condition 45.89 ± 4.89, $t(35)$= 3.28, p<.01). The control condition reported a higher propensity

to become sexually aroused and to be sexually inhibited compared to the stress condition.

Stress induction

For a detailed description of all physiological measures see Oei et al. (2014). A significant Group by Time interaction showed that the TSST led to significant increases in cortisol levels (directly after TSST, $t(34) = -2.02$, $p = .05$), higher heart rates, $F(3, 96) = 3.87$, $p = .01$, blood pressure, $t(35) = -2.12$, $p = .04$, and subjective stress, $t(35) = -4.99$, $p < .001$.

Emotion Regulation Task

Means and standard deviations of subjective ratings of successful instruction completion are shown in Table 1. Regarding the sexual stimuli, there were no differences in ratings of how well they were able to regulate in accordance with the instructions, between the stress condition and the control condition, all *p*s> .50. However, men in the stress condition declared they did better at not-modifying emotional response towards neutral pictures (Neutral-Equal), compared to men in the control condition.

	Neutral-Equal		Sex-Equal		Sex-Down	
	M	**SD**	**M**	**SD**	**M**	**SD**
Control	3.41	0.33	2.98	0.28	2.88	0.32
Stress	3.77*	0.17	3.07	0.47	2.91	0.45

Table 1. Means (*M*) and standard deviations (*SD*) of the ratings of successful emotion regulation completion. Notes: Neutral-Equal: neutral pictures, instruction was do not modify emotional response; Sex-Equal: sexual explicit pictures, instruction was do not modify emotional response; Sex-Down: sexual explicit pictures, instruction was to reappraise the emotional value of the images in order to decrease the emotional impact. * $t(36) = -4.10$, $p < .001$.

Main effects of task

It was expected that activation within reward related structures, such as the NAc and amygdala would decrease during down-regulation of sexual arousal, while activation in frontal control areas would increase. Significant clusters in the contrasts of interest and local maxima are presented in Table 2.

In the contrast Sex-Down vs Sex-Equal four significantly activated clusters were found, with mainly activations in frontal structures. One cluster had its peak activation in the left OFC, with local maxima in the left IFG pars opercularis, temporal pole and frontal operculum cortex (see Figure 2). Other clusters were located in the left precentral gyrus, right lateral occipital cortex and left superior frontal gyrus with a local maximum in the left ACC.

In the Sex-Down vs Neutral-Equal contrast only one cluster was found with its peak activation in the left lateral occipital cortex, encompassing bilateral OFC, bilateral caudate and left thalamus.

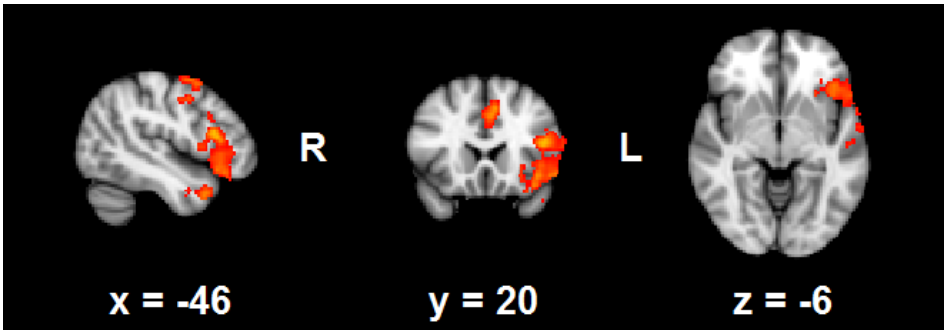

Figure 2. Main effects of the contrast Sex-Down vs. Sex-Equal. Note: (a) Sagittal, (b) coronal and (c) axial view of clusters of voxels ($Z > 2.3$, $p = .05$, cluster-corrected) when contrasting Sex-Down vs Sex-Equal (MNI coordinates, x, y, z = -46, 20, -6). Intensity values in this thresholded zstat map range from 2.3 (red) to 5 (yellow). Voxel size = 2 mm3 in standard space.

Region	Cluster Size	L/R	*x*	*y*	*z*	Z	*P*
• **Local maxima**							
Sex-Down > Sex-Equal							
Orbital frontal cortex	2412	L	-40	24	-10	4.51	<.001
• Inferior frontal gyrus		L	-46	20	16	4.31	
• Temporal pole		L	-48	12	-32	4.16	
• Frontal operculum cortex		L	-38	26	0	4.12	
Superior frontal gyrus	1027	L	-2	10	56	5.40	<.001
• Juxtapositional lobule cortex		L	-2	4	62	4.60	
• Paracingulate gyrus		L	-4	22	38	3.89	
• ACC		L	0	22	26	2.46	
Precentral gyrus	542	L	-52	0	50	4.34	.001
• Middle frontal gyrus		L	-38	4	52	3.05	
Lateral occipital cortex	413	R	44	-70	18	3.99	.004
Sex-Down > Neutral-Equal							
Lateral occipital cortex	54435	L	-42	-82	-2	8.61	<.001
• Orbital frontal cortex		R	34	22	-14	4.46	
• Orbital frontal cortex		L	-34	26	-4	4.94	
• Caudate		R	10	10	4	4.54	
• Caudate		L	-14	10	1	4.61	
• Thalamus		L	-16	-32	0	4.47	

Table 2. Cluster list of significant main effects and local maxima. Note: Z> 2.3, p= .05, cluster corrected. L/R= Left/right in the brain; X,Y, Z = mni coordinates. Voxel size is 2 mm isotropic.

ROI-analyses

To investigate if acute stress affected the ability to down-regulate sexual arousal, independent ROI-analyses were performed in the NAc, amygdala, dorsal ACC, OFC, MFG and IFG triangularis (p< .05, voxel corrected). It was hypothesized that acute stress would affect the down-regulation of sexual arousal by increasing activations in ventral affective brain areas, such as NAc

and amygdala (Oei et al., 2012), while leading to decreased activation in dorsolateral frontal areas such as the MFG. Significant differences in activation between conditions were found for the Sex-Down > Sex-Equal contrast in the right amygdala (p< .02), right dorsal ACC (p< .04), and right IFG pars triangularis (p< .03), with the stress condition demonstrating higher activation in these structures (see Figure 3). There were no significant differences between conditions in activation in NAc, OFC or MFG during the down-regulation of sexual arousal.

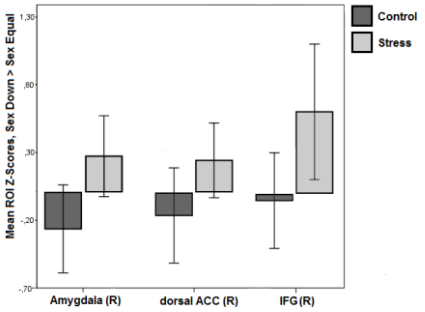

Figure 3. Mean ROI Z-scores (with standard error bars) for the contrast Sex Down vs. Sex Equal for the control and stress condition in the right amygdala, right dorsal ACC and right inferior frontal gyrus (IFG) pars triangularis.

Correlational analyses

To further explore the significant differences between the stress and control condition in down-regulation of sexual arousal, zstats extracted from the right IFG, right amygdala and right dorsal ACC ROIs using Featquery were correlated with the participants' ratings of the ability to regulate sexual arousal. Because the right IFG pars triangularis is known to be involved in successful inhibition of emotion (Aron, Robbins & Poldrack, 2014; Grecucci et al., 2013), it was hypothesized that enhanced activation in the right IFG pars triangularis

would correlate with the perceived ability to down-regulate sexual arousal elicited by the sexual stimuli. For participants in the control condition, activity in the right IFG pars triangularis did not correlate to the ratings of successful down-regulation of sexual arousal, $p = .43$, whereas for participants in the stress condition, activity in the right IFG pars triangularis was significantly correlated to the ratings of successful down-regulation of sexual arousal ($r = .46$, $p < .05$; and when controlled for Neutral ratings $r = .48$, $p < .04$), see Figure 4. Likewise, correlational analyses were performed for the right amygdala and right dorsal ACC, but no significant correlations could be observed (all ps > .13).

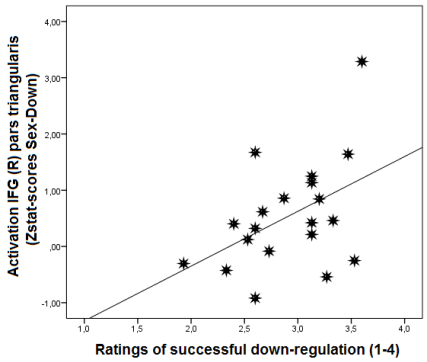

Figure 4. Scatter plot depicting that in the stress condition, greater activation in the right inferior frontal gyrus (IFG) pars triangularis in the contrast Sex-Down significantly correlated with reported greater sexual arousal down-regulation success ($r = .45$, $p < .05$).

10.4. Discussion

The present study investigated the effects of acute stress on the down-regulation of sexual arousal. The results presented here, accord well with previous neuroimaging studies on down-regulation of sexual arousal (Beauregard et al., 2001) and ER in general (Beauregard, 2007; Frank et al.,

2014; Ochsner, 2004; Phan et al., 2005), and extends these previous studies, exploring for the first time whether acute stress modulates brain responses during down-regulation of sexual arousal. First, when participants were instructed to down-regulate sexual arousal, increased neural activation was seen in frontal structures such as the IFG, superior frontal gyrus and frontal pole, and no activity was seen within reward related structures such as the NAc and amygdala. Crucially, acute stress increased activity in the right amygdala, right IFG and right dorsal ACC during the down-regulation of sexual arousal. Moreover, in stressed participants, activity in the right IFG correlated with the perceived ability to down-regulate sexual arousal elicited by the sexual pictures.

Like expected, our data suggest that stress has an impact on the down-regulation of sexual arousal. Although research has shown reduced amygdala activity during cognitive down-regulation (Frank et al., 2014; Kanske et al., 2011; Kim & Hamann, 2007; Ochsner et al., 2004; Townsend et al., 2013) in the present study, acute stress increased activity in the right amygdala during down-regulation of sexual arousal (compared to just watching sexual stimuli). This corroborates research that has demonstrated exaggerated amygdala response under stress (Oei et al., 2012b). This shift of amygdala function toward heightened sensitivity under stress may represent a state of indiscriminate hypervigilance and may correspond to broader dimensions of information processing (e.g. salience, significance, ambiguity, unpredictability, etc.), and may not map specifically onto emotion (Pessoa & Adolphs, 2010, 2011). Although this represents initial survival value in situations where the risk for false negatives in the detection of potential threats should be minimized, speculatively, it might similarly play a role in the development of disorders in sexual motivation, such as hypersexuality. For instance, several studies have demonstrated that activity in the ventral striatum (e.g. NAc) is associated with sexual risk behaviours over time in young adults (Demos et al., 2012; Victor et al., 2015), but results from the study by Victor et al. (2015) suggest that the

expression of ventral striatum-associated sexual risk behaviour is moderated by the magnitude of amygdala activity, especially in men. They found that increased ventral striatum activity is associated with a greater number of sexual partners over time, only in the context of relatively decreased amygdala activity. Speculatively, increased reward sensitivity in combination with low amygdala activity could translate in a heightened drive to pursue immediate (sexual) rewards, but in absence of the ability to recognize and avoid threat. Additionally, individuals who frequently encounter sexual rewarding stimuli when under stress would run the risk of amplified incentive salience of rewards and ultimately addictions (Robinson & Berridge, 1993; Oei et al., 2014). The finding that exposure to acute stress impacts the functional integrity of the PFC (Arnsten, 2009; Arnsten, Wang & Paspalas, 2012), and has deleterious effects on the successful execution of ER (Arnsten, 2009; Raio et al., 2013) may further compromise the ability to regulate sexual arousal in men. However, to date, no controlled experimental studies have investigated emotion down-regulation and functional connectivity in subjects with hypersexuality, which could elucidate trait-level dysfunction in suggested key neural circuitry. The current study did not investigate if the activity in the ventral striatum was also associated with sexual risk behaviour, and whether this was moderated by the magnitude of amygdala activity, but recent research from our lab demonstrated that acute stress-induced cortisol elevations mediate NAc activity during the subconscious processing of sexual stimuli (Oei et al., 2014) and that high stress-induced cortisol responses were negatively correlated with amygdala responses during emotional inhibition (Oei et al., 2012b). Altough no differences were seen in actitvity in the NAc between control and stressed participants in the present study (but see Oei et al., 2014), the above suggest that individuals with a certain phenotype (increased NAc activity as a result of high stress induced cortisol, in combination with low amygdala activity) may be vulnerable for

developing problematic sexual behaviour. However, as has become clear, more research is warranted.

It is thought that ventrolateral PFC dysfunction may explain the failure to modulate or inhibit limbic regions underlying affect, including the amygdala. The right IFG pars triangularis is known for stopping action, and to be specific, for stopping action tendencies (Aron, Robbins & Poldrack, 2014). Results from the present study might point at the contribution of the ventrolateral PFC in controlling sexual arousal. Sexual stimuli evoke automatic (approach) responses, and the right IFG pars triangularis might be involved in suppressing these tendencies (Both et al., 2005; Dekker & Everaerd, 1989; Lang, 1971), especially when this suppressing is further compromised by the induction of stress. Results from the correlational analyses suggest that, speculatively, additional right IFG pars triangularis resources were specifically recruited as the acute-stress induction costs for providing top-down control of sexual arousal increased. However, another explanation might be that this enhanced right IFG activation may reflect increased emotional coping mechanisms, as a result of high stress (Anderson, 1976; Yuen et al., 2009).

Research has shown that dorsal regions of the ACC are involved in appraisal and expression of negative emotion, but also in reward-based decision making (Bush et al., 2002; Etkin, Egner & Kalisch, 2011). Moreover, the dorsal ACC has strong interconnections with lateral PFC (Bush, Luu & Posner, 2000). Therefore, the enhanced recruitment of the dorsal ACC in the stress condition while down-regulating sexual arousal may reflect the guidance of behaviour by evaluating motivation and encoding reward values, which may then influence attention allocation, and even motor preparation and motor responses (Bush et al., 2002).

Although this study highlights the potential deleterious effect of acute stress in controlling sexual arousal, there are some limitations of this study that must be considered before definitive inferences can be made. First, an

important caveat to consider when interpreting these findings is that on each trial subjective and physiological sexual desire or arousal was not assessed directly and assessment of regulation success was limited to self-report. Therefore, firm conclusions about patterns of brain activation and actual levels of sexual arousal and desire cannot be draw. Second, the current study sample only comprised of healthy sexually functional men. Therefore, we can only speculate about the suggested key neural circuitry involved in hypersexual behaviours. Moreover, research suggests that men and women may differ in their ability to regulate emotions (Whittle, 2011), including sexual arousal (Brom et al., 2015). Since, imaging studies in women on the regulation of sexual arousal are lacking in the literature, future research on the influence of stress on the regulation of sexual arousal in women is warranted. Moreover, the present study only investigated the reappraisal strategy. Different strategies, including attentional control (e.g. distraction) may be adopted to achieve successful emotion down-regulation. Therefore, the effect of the emotional down-regulatory strategy in the present study does not reflect the complexities of the emotion regulation repertoire (Aldao, 2013).

Clinically, these data might imply that if patients with hypersexuality are impaired in the ability to suppress the impact of sexual material, this might reflect a decreased ability -at least in some patients- of the ventrolateral PFC to suppress a pathological increased NAc response to sexual stimuli as a result of experienced stress in combination with a relatively decreased amygdala activity (Demos et al., 2012; Oei et al., 2014; Victor et al., 2015). Stress has been related to an increased vulnerability to develop drug intake or relapse (Sinha, 2008). This makes clear that future studies using the current tasks should explore possible deficits in this proposed neural circuitry in patients with substance use disorders or sexual addiction. Nevertheless, targeting the effects of stress on the regulation of sexual arousal might be helpful in predicting individual susceptibility to relapse. Furthering our understanding of how stress may impair

ER may lead to better interventions that foster resistance to stress-induced regulatory impairments and offer better treatment options for clinical populations.

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Chapter 11

General Conclusion & Discussion

11. General Conclusion & Discussion

The central question of this thesis is whether and to what extent human sexual arousal response is susceptible to lower-level control processes like basic associative learning processes (i.e. classical conditioning) and related phenomena (e.g. extinction and renewal), and whether higher-level control processes such as cognitive emotion-regulation can also influence sexual incentive learning in healthy participants. In addition, we were interested in the effects of acute-stress on the cognitive regulation of sexual arousal. In this final chapter the empirical findings of this thesis will be summarized, integrated and critically discussed, starting with a detailed summary of the role of basic learning principles in (animal and) human sexual arousal and behaviour. Hereafter, challenges and clinical implications will be discussed, concluding with future research direction.

11.1. Lower-level Control Mechanisms in Sexual Arousal

11.1.1. The Role of Basic Learning in Sexual Behaviour

Due to the fact that historically, theories of emotion have not given much consideration to sex there is only limited empirical research on human sexual incentive learning, especially in women (Toates, 2014). Nevertheless, the treatment demands for disorders in sexual motivation, such as female sexual interest/arousal disorder, or hypersexuality and paraphilia-related disorders call for research investigating the possible mechanisms underlying sexual arousal response and sexual motivation (Kafka, 2007; Mercer et al., 2003; Ter Kuile, Both & van Lankveld, 2010; West et al., 2008). In chapter two a thorough review is given of animal and human studies that examined the role of classical

conditioning, learning, and DA in sexual behaviour, which were published or in press before October 2013. In this chapter animal and human sexual learning is tied into a more general framework of incentive motivational theory (Bindra, 1974; Pfaus, Kippin & Centeno, 2001; Singer & Toates, 1987). Also the studies on sexual learning in animals were included in this review because a role for learning in the sexual behaviour of animals also has profound implications for our understanding of human sexual arousal and the development of disorders in sexual motivation. The animal research described in this review chapter shows robust and direct effects of sexual conditioning processes on sexual behaviour, such as partner- and place preferences. Moreover, in a wide range of taxa, positive as well as negative experiences can modify sexual responses in animals. Intriguingly, although arbitrary (i.e. auditory, visual or olfactory) CSs are effective in eliciting sexual CRs in male and female animals, a greater CS-US similarity appears to elicit CRs that seem to be highly resistant to extinction, especially in male animals (Domjan et al., 1988; Krause et al. 2003; Rescorla & Furrow, 1977). Second, animal studies have shown that increased DA concentrations in the NAc are essential for the acquisition of reward learning, including sexual reward learning (López & Ettenberg, 2002; Pfaus et al., 1990; Robinson & Berridge, 2003). Animal studies showed that a state of sexual reward (i.e. ejaculation in male rats, or paced mating or vaginocervical/clitoral stimulation in female rats) is a powerful mediator of incentive formation and enhancement, which depends on DA functioning (e.g. Kippin et al., 2003; Mermelstein & Becker, 1995; Paredes & Alonso, 1997; Parada et al., 2011, 2013; West et al., 1992). Although research on human sexual incentive learning has lagged substantially behind that of animal sexual functioning, there is evidence that in humans, classical conditioning can also augment or diminish subjective and genital sexual arousal in men and women. The earlier sexual conditioning studies -mainly in men- within this field are plagued by methodological confounds, however recent well-controlled experimental

research shows that human sexual arousal can be conditioned (Both et al., 2008a,b, 2011; Hoffmann, Janssen & Turner, 2004; Klucken et al., 2009). Nevertheless, it should be mentioned that also a considerable number of these more recent studies comprise only small study samples, or sample sizes too small to make sound group and gender comparisons (e.g. Hoffmann, Janssen & Turner, 2004; Hoffmann et al., 2012; Lalumière & Quinsey, 1998; Plaud & Martini, 1999). Taking this in consideration, results suggest there is some prudent evidence that in men, US-CS similarity is an important factor in sexual incentive learning (Hoffmann, Janssen & Turner, 2004), and these results are suggestive of a 'prepared' or 'already learned' link between sexual stimuli and genital responses as has been proposed by some models of sexual arousal (e.g., Janssen et al., 2000). Additionally, it is suggested that conditioned sexual evaluative learning effects seem highly resistant to extinction (Both et al., 2008a,b, 2011). Unfortunately, although it is assumed that the involvement of limbic reward circuitry is also crucial for human sexual incentive learning (Berke & Hymann, 2000; Di Chiara, 1999), no studies have investigated the role of DA in human sexual learning, and only little imaging studies on human sexual conditioning have been reported (Klucken et al., 2009, and see also Klucken et al., 2014). Nevertheless, in those studies conditioned activation was seen in reward structures (e.g. NAc). From the above, it can be concluded that sexual behaviour in animals and human sexual arousal can be classical conditioned. This sexual incentive formation and enhancement is mediated by DA functioning in animals.

11.1.2. The Role of DA in Human Sexual Reward Learning

Although the dopaminergic reward system has been implicated to be involved in the acquisition and expression of learned appetitive behaviours, and abnormality in this system has been shown to play an important role in the

aetiology and pathophysiology of various disorders, including substance use disorders and (behavioural) addictions (Dominguez & Hull, 2005; Fields et al., 2007; Schultz, 2007; Richard et al., 2012; De Jong et al., 2015; Dunlop & Nemeroff, 2007; Root et al., 2015), the role of phasic DA signalling in sexual incentive learning in humans remains largely unknown, while facilitation as well as impairment thereof is relevant in the context of treatment of sexual motivation disorders. In chapter three, making use of a double-blind, parallel-conditions, placebo controlled design, it was investigated whether DA antagonism would attenuate classical conditioning of sexual response in women. Results from this study demonstrated that DA receptor antagonism reduced sexual stimulation-induced genital sexual arousal, emphasizing the importance of DA availability in unconditional responding to sexual stimulation. This is in accordance with previous work that showed that DA systems are involved in human sexual reward signalling (Both et al., 2005; Georgiadis & Kringelbach, 2012; Oei et al., 2012). However, quite intriguing but contrary to the expectations, is the finding that DA down-regulation did not seem to affect subsequent (slight conditioned genital response and) conditioned subjective sexual arousal. But it should be mentioned that only very weak conditioned genital responding was seen in both conditions. Nevertheless, the administration of haloperidol did not seem to affect the perceived pleasantness or sexual arousability of the US, or the magnitude of conditioned subjective sexual arousal. The lack of a difference in genital conditioned responding between the Placebo and Haloperidol condition makes future replication studies warranted. Second, since the study sample exclusively comprised women, the role of DA in mediating associative sexual learning in men is still to be determined. Research has shown that gender differences in the number of DA neurons are influenced by several factors, including sex chromosome complement (Lombardo et al., 2012), the presence of the sry gene (Dewing et al., 2006) and gonadal hormones. Therefore it is conceivable that

gender differences in sexual conditionability and underlying neuromodulatory systems do exist. However as has become clear, more research is needed to be conclusive about this.

11.1.3. Sexual Evaluative Learning Effects

As has become clear in chapter three, in animals there is evidence that conditioned responses to sexually relevant CSs seem to be resistant to extinction (Domjan et al., 1988; Krause et al. 2003; Rescorla & Furrow, 1977). In addition, results from the sexual conditioning studies by Both and colleagues (Both et al., 2008a,b, 2011) showed that conditioned subjective affect did not extinguish significantly during an extinction procedure, suggesting resistance to extinction of sexual learning effects, also in humans. These results are in line with research on evaluative conditioning: although extinction procedures do eliminate the expressions of US expectancy, extinction procedures do not change the expressed valence of a CS, and as a result, exposure treatment is often unsuccessful in reducing acquired subjective (dis-) likes (Baeyens, et al., 1992; de Houwer, et al., 2001). To investigate a possible resistance to extinction of sexual learning effects in men and women, two studies were conducted in which extensive extinction trials were used. In chapter four the experimental study on extinction of appetitively conditioned responses is reported, and likewise in chapter five a parallel study on extinction of aversively conditioned sexual responses is presented.

In chapter four, evidence is found for the claim that appetitively conditioned sexual evaluative learning effects are rather difficult to modify through the procedure of extinction, although no evidence was found for the claim that these effects are indeed resistant to extinction. In this study, genital vibrostimulation served as US and neutral pictures as CSs. Although the

extinction procedure eventually reduced conditioned subjective affect and sexual arousal towards the CS+, these evaluative learning effects were relatively persistent, as evidenced by conditioned responses even after extensive extinction trials. Moreover, after extinction, behavioural approach was investigated with a joystick Approach-Avoidance Task (AAT), a task that mimics actual approach and avoidance (Cousijn, Goudriaan & Wiers, 2011). The results from this task demonstrated that the pairing of the CS+ with the sexual vibrotactile stimulus did still result in slight approach tendencies towards this CS+ in men and women, suggesting the CS+ retained sexual affective value to elicit approach even after a very extensive extinction phase. However, it is important to keep in mind that this effect only approached a conventional level of statistical significance. Contrary to the expectations, no conditioned genital responses was observed in men and women, but this was thought not to hamper any conclusions about the persistence of sexual evaluative learning effects.

In the parallel aversive sexual conditioning study that is reported in chapter five, a different pattern was seen. This study provided evidence that an extinction procedure is well capable of modifying aversively conditioned sexual evaluative learning effects, at least in healthy men and women. Making use of a painful stimulus as US and erotic pictures as CSs, attenuated genital blood flow was seen on the first extinction trials in women in response to the erotic picture that was previously paired with the painful electric stimulus. However, no such conditioned genital response in men was observed. Likewise, only women rated the CS+ as slightly less sexually arousing compared to the erotic picture that was never followed by painful stimulation, while men did not demonstrate attenuated subjective sexual response in response to the CS+ during the first trials of the extinction phase. Crucially, subjective affective value was modulated by repeated association of the erotic stimulus with pain. Men and

women rated the erotic picture that was paired with pain stimulation as more negative than the erotic picture that was not paired with pain. However, the difference in affective evaluation of the CS+ and the CS decreased over time during the extinction phase, suggesting that aversively conditioned responses are not resistant to extinction. Furthermore, no conditioned behavioural avoidance tendencies were seen towards the CS+ after the extinction phase. These findings are quite intriguing, especially considering that exact the same procedure was used as in chapter four, with the USs and CSs as the only differences. The results suggest that appetitive and aversive sexual extinction learning encompass distinct processes and are not organized in the same fashion. We will return to this issue shortly.

To conclude, the presented results raise doubt about the claim that, unlike other forms of classical conditioning, evaluative sexual conditioning is resistant to extinction. Analyses in both -appetitive and aversive- sexual conditioning studies showed that unpaired presentations after the CS-US trials reduce the magnitude of conditioned responses, including the magnitude of sexual evaluative learning effects. Hence, sexual evaluative learning is sensitive to extinction. Nevertheless, results suggests that extinction of sexual evaluative learning effects may occur at a slower rate than in other forms of associative learning (i.e. signal learning) (Hofmann, de Houwer, Perugini et al., 2010), especially in an appetitive conditioning paradigm. Possible clinical implications hereof will be discussed later on.

11.1.4. Context Specificity of Sexual Extinction Learning

Although the evidence regarding renewal in human learning has accumulated in recent years, studies on renewal of sexual conditioned responses were lacking in the literature, despite the possible important implications for exposure-based

treatment strategies for learned maladaptive sexual responses. In chapter six an experiment is reported on extinction and renewal of appetitively conditioned sexual responses in sexually functional men and women. In this study an ABA renewal paradigm was used, and different contexts were obtained by manipulating the lighting conditions in the experimental room. It was predicted that participants in both conditions (AAA and ABA) would show conditioned sexual responding after acquisition trials, which was expected to gradually decrease. As an index of renewal, it was predicted that upon a context change after extinction, only the ABA condition would show recovery of conditioned responding on the test trials as compared to the last extinction trial. Results from this study are in favour of Bouton's theory of context dependency of extinction and renewal of conditioned responding (Bouton, 2004). Changing context after an extinction procedure resulted in a significant increase of subjective affect and subjective sexual arousal in women and increased US expectancy ratings to CS+ as compared to CS- in both men and women (ABA condition), whereas no such recovery was observed in the absence of a context change (AAA condition). However, no evidence for renewal was found for genital measures in both sexes. For men, this could be explained by the fact that genital conditioning effects were not obtained, and in women extinction of conditioned genital responding was not completely ascertained during the extinction phase, making it harder to detect renewal of conditioned responding. After the experimental conditioning procedure, men and women differed in implicit approach tendencies towards the stimulus that was paired with vibrostimulation, with women significantly faster approaching the CS+ than men. In women the CS+ elicited a more robust sexual arousal response as compared to men. This means that in women, the conditioned sexual response translated into subjective experience, physiological measures and eventually also in action tendencies. Results from this study make clear that sexual arousal or the expectation of sexual reward can come under stimulus control by

contextual cues associated with states of sexual reward. With other words, these results emphasize the importance of sensitivity to context (changes) in sexual learning.

Evidently, it is impossible to cover all sorts of situations or stimuli in therapy (i.e. extinction) sessions, meaning there will always be a certain risk for patients to relapse when confronted with a particular object, situation or mental state. Indeed, it is suggested that context specificity of extinction impairs its ability to generalize extinction to the context in which the problematic behaviour is experienced. Therefore a highly promising perspective is to focus on processes that modulate contextual processing during extinction procedures, since any pharmacological agent that that can render extinction context independent may provide an innovative method to reduce cue-induced relapse in the treatment of problematic reward-seeking behaviours. In chapter seven the effect of a single dose of (the NR1 NMDA receptor subunit agonist) D-cycloserine (DCS) on the reduction of context specificity of extinction of sexual reward-associated cues in humans was investigated. The design consisted of sexual conditioning in context A and extinction in context B. It was hypothesized that administration of DCS after an extinction procedure would enhance extinction of conditioned sexual responses, reflected by a loss of conditioned genital and subjective sexual responding elicited by reward-conditioned cues in participants receiving DCS, even outside the extinction context, compared to participants in the placebo condition on a recall test 24h later. In this study it is demonstrated that DCS indeed affects extinction's fundamental context specificity in women, at least in an (ABAB) appetitive sexual conditioning paradigm, since DCS enhanced extinction of conditioned responses also in the original acquisition context. These results are highly interesting, especially when there is no a priori reason to believe that a drug that enhances extinction learning will change the nature of extinction learning

qualitatively (Todd et al., 2014). However, since no imaging results were obtained during the sexual conditioning and subsequent extinction procedures, we can only speculate about possible underlying neural mechanisms (Torregrossa et al., 2013).

To conclude, results from chapter six indicate that an extinction procedure does not erase conditioned sexual associations in humans but instead involves new learning that is context dependent. Results from chapter seven suggest that in healthy sexually functional women, DCS makes sexual extinction memories context-independent and prevents the return of conditioned sexual response. NMDA receptor glycine site agonists may be potential pharmacotherapies to enhance sexual extinction memory, herewith reducing the motivational impact of sexual reward-associated cues, and to prevent relapse in sexual motivation disorders with a learned component.

11.2. Higher-level Control Mechanisms in Sexual Arousal

Obviously, humans are not simply driven by external sexual cues and incentives. We have the ability to process stimuli and situations in a deliberate, controlled and often conscious way. Our cognitive abilities allow us to determine stimulus meaning and predispose action (Frijda, 1986; LeDoux, 2012). Janssen and Bancroft (2007) have suggested that the response following exposure to a sexual stimulus depends on automatic bottom-up appraisal and response-generation processes as well as on effortful top-down regulatory processes. Meaning, our cognitive ability allows us to influence and alter emotions by using thoughts. In this way, cognition can be tuned in the service of generating more adaptive emotional reactions. Hitherto, most insights in cognitive emotion regulation come from research on maladaptive responses and behaviours as seen in drug addictions, anxiety disorders or depression, and

research on the regulation of positive (i.e. pleasant-valenced) emotions such as sexual arousal is extremely scarce in the literature (Carl et al., 2013; Beauregard, 2007). Moreover, literature on sex differences in the regulation of positive emotions was lacking in the literature. Research on the regulation of particularly negative emotions (Gross, 2007; Mak et al., 2009; McRae et al., 2008) indicated that women tend to use more emotion-focused strategies, while men are thought to use more efficient cognitive (rational) strategies (Whittle et al., 2011). Despite the hypothesized importance of understanding how to regulate or control the positive feelings associated with sexual reward expectation, and the fact that insight in the mechanisms of these cognition-emotion interactions can help in the development of effective CBT interventions, research on the influence of emotion regulation strategies on the expectation of sexual reward was lacking in the literature. In chapter eight and nine the influence of higher-level control mechanisms on sexual conditioned responses and extinction thereof in healthy men and women was examined.

In chapter eight, making use of a differential appetitive sexual conditioning paradigm, a cognitive down-regulation condition was compared with a control condition in men and women. It was demonstrated that the deployment of a cognitive emotion down-regulation strategy effectively enhanced extinction of conditioned affective value and subjective sexual arousal in men as compared to men in the control condition. Intriguingly, in women the deployment of the down-regulatory strategy resulted in overall higher ratings of affective value and subjective sexual arousal towards both CSs. In women, the cognitive strategy did however result in attenuated approach tendencies towards conditioned stimuli that predicted potential sexual reward (i.e. the CS+). In men, the cognitive strategy did not result in attenuated approach tendencies towards the CS+. Crucially, the deployment of a cognitive down-regulation strategy did not result in decreased conditioned genital sexual

arousal, or subjective affect and sexual arousal in both sexes. In addition, US expectancy was not affected at all by the cognitive strategy, in men and women.

In chapter nine it was investigated whether a cognitive up-regulatory strategy can efficiently increase sexual arousal elicited by sexual reward-conditioned cues in healthy men and women. In this study it was demonstrated that the deployment of the emotion up-regulatory strategy did not seem to have any effect on conditioned genital responding in men and women. However, the cognitive strategy did increase unconditioned genital responses in both sexes. Additionally, the deployment of the cognitive up-regulatory strategy seemed to result in enhanced resistance to extinction of conditioned genital responding in women. Regarding the subjective measures, results indicate that in men conditioned affective value can be up-regulated by cognitive strategies, whereas in women no such effect was observed. On measures of subjective sexual arousal and US expectancy the emotion up-regulatory strategy did not seem to affect conditioned responding or extinction thereof, in both sexes. The emotion up-regulation strategy also did not result in increased approach tendencies towards the CS+ in men and women.

Results from these studies indicate that sexual arousal can be modulated in line with participants' regulatory goals, despite mixed results for men and women. Compared to women, men appear more effective in emotional down-regulation of sexual arousal, whereas top-down up-regulation can influence conditioned sexual responses or extinction thereof in both sexes. Intriguingly, US expectancy was not affected by either cognitive regulatory strategy. Results from those studies indicate that emotion regulation strategies do not seem to be equally effective on all sexual responses (i.e. behavioural, affective value, physiological, US expectancy).

11.2.1. The Influence of Acute-Stress on High-Level Top-Down Control

As has been elucidated in chapters one and two, the dopaminergic pathways are widely known for their involvement in the signalling of rewarding stimuli, but also aversive events including acute stress, can activate the dopaminergic neurons in the brain reward system (Kringelbach & Berridge, 2009; Pruessner et al., 2004; Oei et al., 2014). The effects of stress are thought to be mediated by neuroendocrine responses to acute stress exposure (i.e. increased cortisol levels) that impact not only subcortical reward structures (Oei et al., 2014) but also the functional integrity of PFC (Raio et al., 2013). Because the relationship between the physiological stress response and the cognitive control of sexual arousal had not been examined, in chapter ten the influence of acute-stress on deliberate emotion regulation during the processing of sexual stimuli was investigated. Making use of functional magnetic resonance imaging (fMRI) we tried to shed light on the effect of acute-stress on within-subject functional activity in brain regions associated with sexual reward (e.g. the amygdala and NAc) and frontal regions during cognitive down-regulation of sexual arousal. Hereto, healthy sexual functional men were randomly assigned to an acute stress or control condition.

It was expected that activation within reward structures, such as the NAc and amygdala would decrease consistent with the goal of down-regulation, while activation in frontal structures would increase respectively (Ochsner et al., 2004), and indeed such a reciprocal pattern was seen for the whole brain analyses. When participants were instructed to down-regulate sexual arousal, increased neural activation was seen in frontal structures, such as the inferior and superior frontal gyrus and frontal pole, and no activity was detected in the amygdala. Crucially, acute-stress increased activity in the right amygdala, right inferior frontal gyrus and right dorsal ACC during the down-regulation of sexual arousal, compared to when participants were instructed just to just watch

the sexual stimuli. Moreover, in the acute-stress condition, activity in the right inferior frontal gyrus pars triangularis was significantly correlated to the ratings of successful down-regulation of sexual arousal, suggesting that additional right inferior frontal gyrus pars triangularis resources were specifically recruited as the acute-stress induction costs for providing top-down control of sexual arousal increased.

These results corroborate previous research on the regulation of sexual arousal (Beauregard et al., 2001) and provide evidence for the view previously proposed that regulation of sexual arousal depends on a neural circuit in which frontal cortical areas mediate the cognitive modulation of sexual responses generated at a subcortical level. Moreover, the results are in favour of the assumption that stress may complicate the successful cognitive down-regulation of sexual arousal, as evidenced by the increased neural activation in the right amygdala, right dorsal ACC, and right inferior frontal gyrus pars triangularis. Results from the present study indicate that acute-stress is an important threat for the successful regulation of sexual arousal.

11.3. Factors Influencing Control Mechanism in Sexual Arousal

The above makes clear that sexual arousal or the expectation of sexual reward can come under stimulus, contextual, and cognitive control. In this paragraph important factors influencing lower-level control processes in sexual incentive learning are discussed. Hereafter, we will tap into the factors involved in higher-level control processes.

11.3.1. Lower-Level Control Factors in (Conditioned) Sexual Arousal

In chapter two it is suggested that CS-US similarity plays an important factor in animal sexual conditioning, and that conditioned responses toward sexually relevant CSs are highly resistant to extinction (Domjan et al., 1988; Krause et al. 2003; Rescorla & Furrow, 1977). It is thought that those 'prepared' associations are acquired more easily and that additionally these associations are thought to obey different laws of learning than nonprepared associations do. However, as mentioned earlier, this theory of preparedness is not undisputed, and alternative theories, such as selective sensitization (i.e. a pre-existing response tendency is activated by a perceived threat; Lovibond, 1993) or biases in the processing of information about certain stimuli rather than phylogenetically based associative predispositions have been put forward (Davey, 1995). In spite of its controversy, results from the appetitive and aversive sexual conditioning studies reported in the chapters four and five respectively, can be explained by such a model of preparedness or selective sensitization. To clarify, in the aversive conditioning study, the association between sexual stimuli (erotic CSs) and the suppression of sexual arousal (painful US) (i.e. CS-US dissimilarity) may explain the sensitivity to extinction of aversively conditioned sexual responses, especially since healthy sexually functional men and women participated in this study. Research has demonstrated appetitive - aversive interactions in DA neurons in the brain reward system: when a neuron is excited by an aversive CS it is inhibited by an appetitive CS or vice versa (Matsumoto & Hikosaka, 2009; Bouton & Peck, 1992; Nasser & McNally, 2012). In addition, recruitment of the relevant motivational system (appetitive vs aversive) is dependent on the US. Painful stimulation (e.g. electric shock) can selectively activate the aversive system, whereas sexual stimulation (e.g. genital vibrostimulation) can selectively activate the appetitive system. However, since erotic pictures were used as CSs in the aversive conditioning study (chapter five), these pictures most likely

automatically recruited the appetitive motivational system in healthy men and women. In addition, the painful stimulation that served as US most likely recruited the aversive motivational system. Since the two motivational systems oppose each other, a CS which excites one motivational system will inhibit the other. In other words, a conditioned excitor of one motivational system is functionally equivalent to a conditioned inhibitor of the other, and prior appetitive sexual learning could have interfered or augmented sexual aversive learning (Nasser & McNally, 2012). This mechanism may explain why the aversively conditioned sexual responses do not seem to be resistant to extinction, at least in healthy sexually functional men and women. In the appetitive conditioning study neutral pictures were used as CSs, and as a consequence, it is thought that only the appetitive motivational system was recruited by the US, and no prior (aversive) learning interfered with CR acquisition. This also suggests that humans are not only capable in coding events and/or stimuli that are related, but also in coding *how* these are related. The persistence of evaluative learning effects can therefore be explained by the assumption that once a stimulus has been categorized as potential cause of an aversive or appetitive outcome, individuals fall back on their prior propositional knowledge about causal relations, including the general knowledge that causes tend to have additional effects (De Houwer, 2009).

Moreover, although results from studies in this thesis contribute to the accumulating evidence (Both et al., 2008a,b, 2011) that women's genital response can be appetitively conditioned to initially neutral stimuli, at least, when making use of a tactile US, results from studies discussed in this thesis do not support such a straightforward mechanism in men. In the chapters four and six, making use of two neutral pictures of pictorial faces as CSs, no evidence for conditioned genial arousal response could be observed in men. In chapter eight, now making use of sexually relevant stimuli as CSs, conditioned genital

responses were detected in men. Although this thesis was not specifically aimed at investigating the difference between sexually relevant versus sexually irrelevant stimuli in sexual conditioning, and a comparison between results from those studies is not straightforward because of the difference in used design (and one needs to keep in mind that in chapter nine no conditioned genital responses could be observed in men despite making use of sexually relevant CSs), speculatively, it could be that men are less susceptible to sexual learning to cues that differ too much from their developed preference (Coria-Avila, 2012; Pfaus, Kippin & Centeno, 2001). Therefore it seems that combination of a tactile sexual US and neutral CSs is not sufficient to elicit conditioned genital responding in men, whereas the combination of a tactile sexual US and sexually relevant CSs is capable of triggering such a response. However, it should be mentioned that making use of sexually explicit visual stimuli as US, conditioned genital responses towards an initial neutral CS (a penny jar) were observed by Plaud and Martini (1999), although their sample size comprised of only nine subjects. Nevertheless, it could very well be that men and women differ in sexual learning, with women having more erotic plasticity, once sexual preferences are established (Baumeister, 2000; Coria-Avila, 2012; Pfaus, Kippin & Centeno, 2001). Based on Hoffmann, Janssen and Turner (2004) genital results from chapters four and eight might indeed be seen from such a perspective, since in their sexual conditioning study, women showed conditioned arousal to the sexually irrelevant rather than the relevant CS, whereas men receiving conscious presentations of the CS showed more evidence of conditioned sexual arousal to the sexually relevant CS (i.e. abdomen) than to the irrelevant CS (i.e. gun). Future well-powered research, incorporating sexually relevant and sexually irrelevant stimuli in one design is needed before any firm conclusions about 'prepared' (or 'already learned') sexual associations and possible sex differences therein can be drawn.

The studies in this thesis are the first to investigate whether initially neutral cues can elicit approach tendencies through their mere pairing with a sexually rewarding outcome, and likewise whether initially sexual cues can elicit avoidance tendencies through the association with a painful outcome. The processing of emotionally competent stimuli results in physiological changes that prepare an organism for action (Both, Everaerd & Laan, 2005). In case of threatening stimuli avoidance behaviour will be activated, and conversely, in case of attractive (sexual) stimuli appetitive approach behaviour will be triggered. In this thesis it was hypothesized that stimuli that elicit emotional arousal will facilitate action tendencies, relative to neutral, low arousal stimuli. In chapters four, five and six, additionally to the experimental sexual conditioning procedure, a stimulus response compatibility task was included to assess implicit approach and avoidance tendencies towards the CSs. Although in the chapters four and five, this Approach Avoidance Task was administered only after extensive extinction trials, results from chapter six indicate that conditioned female sexual response translated into subjective experience, physiological measures and in action disposition.

11.3.2. Higher-Level Control Factors in (Conditioned) Sexual Arousal

To continue on the topic of conditioned action tendencies, results from chapter eight indicate that a cognitive emotional down regulatory strategy can result in attenuated approach tendencies towards conditioned stimuli that predicted potential sexual reward (i.e. the CS+). Like discussed in chapter one, contemporary emotion theories propose that sexual arousal, like any emotion, is a composite of subjective experience, physiological activity, and action disposition (Frijda, 2010). Moreover, it is also proposed that emotions are primarily action tendencies that are reflected in physiological activity and subjective response. In such a framework, the fact that a CS elicits sexual

arousal response after pairing with a sexually rewarding US implies that the CS also elicits an approach tendency: the approach tendency installed through Pavlovian reward learning is translated into overt action. Additionally, as chapter eight suggests, higher-level control mechanisms can also influence these tendencies. Moreover, results from chapters eight and nine suggest cognitive regulatory strategy mainly operate on physiological measures of sexual response and valence, leaving the more cognitive aspects (US expectancy) of conditioning intact. Research indeed suggests cognitive regulatory effects on US expectancy are not to be expected (Blechert, et al., 2015).

Results from chapter ten are in favour of the assumption that stress may complicate the successful cognitive down-regulation of sexual arousal, as evidenced by the increased neural activation in the right amygdala, right dorsal ACC, and right inferior frontal gyrus pars triangularis. Research has demonstrated that the dorsal regions of the ACC are involved in threat appraisal and expression of negative emotion (Etkin, Egner & Kalisch, 2011; Ochsner & Gross, 2005), as well as in reward-based decision making (Bush et al., 2000). Moreover, research suggests that a disturbance in neural activity in right dorsal ACC may account for emotional dysregulation (Beauregard, Paquette & Le`vesque, 2006). The existing literature and results from the imaging study in this thesis, allows for speculation about the influence of acute-stress on cognitive down-regulation of sexual arousal, with the initial motivational value of the sexual stimuli being calculated in the amygdala, but being further maintained and updated in reward structures such as the NAc and right dorsal ACC, and being controlled by frontal structures. It seems that acute-stress requires enhanced activation of the right inferior frontal gyrus pars triangularis, in order to successfully down-regulate sexual arousal. The inferior and superior frontal gyrus, have been implicated in top-down control, especially in the inhibition or stopping of inherent response tendencies (Aron, Robbins&

Poldrack, 2014). Additionally, other research suggests (Raio et al., 2013) that acute-stress impairs successful regulation of negative emotions. This, and results from the imaging study in this thesis suggests that acute-stress may impair cognitive regulation of emotion, including sexual arousal. Moreover, the results from this study suggest an important paradox: top-down regulation may be compromised in controlling sexual arousal precisely when such control is needed most, especially since aversive events including acute stress (Oei et al., 2014), can activate the dopaminergic neurons in the brain reward system, resulting in increased bottom-up subcortical responses. Moreover, it is important to keep in mind that, as stated before, the principles underlying cognitive regulation also form the basis of CBT. Therefore, the success of CBT relies on the availability of cognitive resources and intact executive function (Heatherton & Wagner, 2011; Hofmann, Schmeichel & Baddeley, 2012; Ochsner, Silvers & Buhle, 2012). The proposed regulatory difficulties described are also consistent with theories of self-regulation failure (Heatherton & Wagner, 2011), that describe self-regulatory capacities as a limited resource that may become weak and depleted when exposed to negative emotions (induced by for instance acute-stress). Derived from this model, regulatory capacities rely on top-down prefrontal control and may be weakest when frontal functioning is impaired, and/or when subcortical regions involved in the automatic emotional response behaviour are enhanced (Oei et al., 2014).

11.4. Gender Differences

11.4.1. Lower-level Control Processes

All studies in this thesis were conducted in healthy sexually functional volunteers, and all studies, apart from the chapters three, seven and ten, included men and women to explore possible gender differences in sexual

conditionability and cognitive regulatory strategies. Results from the studies in this thesis oppose the existing idea that men are more receptive to sexual conditioning than women (Pfaus, Kippin & Centeno, 2001). Although there are some differences observed between men and women in the studies in this thesis, these may not reflect pure gender differences in sexual conditionability per se, but may also be explained by differences in sample size and US effectiveness. Moreover, genital responses of men and women do not lend themselves to be compared directly. Research has shown that for men, more than for women, visual stimuli preferentially recruit an amygdalo-hypothalamic pathway (Hamann et al., 2004). In addition, research also demonstrated that in men, vibrotactile stimulation alone produces the lowest level of genital and subjective sexual arousal compared to erotic film (Rowland & Slob, 1992). Therefore, it remains the question to which extent present results can be generalized to make claims about sexual learning in general, and gender differences therein. It is well possible that making use of visual erotic stimuli as US, a different pattern may be seen. But for now, results from earlier sexual conditioning studies in humans discussed in chapter two, combined with the results from the experimental studies presented in this thesis, provide not enough evidence to support the claim that men and women do differ in basic sexual learning. Nevertheless, the widely held view is that women are more sensitive to variations in social and cultural factors (i.e., exhibit more 'erotic plasticity') compared to men (Baumeister, 2000; Toates, 2009, 2014). It is thought that in women, a sexual stimulus triggers a wider range of cognitions as compared to men (Laan & Janssen, 2007; Toates, 2014). Therefore it is suggested that women's sexual motivation and arousal might be more strongly controlled by cognitive factors, whereas men's sexual motivation tends to be more strongly controlled by stimulus factors.

11.4.2. Higher-Level Control Processes

Hitherto, it is assumed that women may use less efficient cognitive strategies in the regulation of emotions compared to men (Whittle et al., 2011). But it is important to keep in mind that most –if not all- of these results come from studies that investigated the regulation of particularly negative emotions (Mak et al., 2009; McRae et al., 2008; Gross, 2007). Results from chapter nine, do not suggest that men and women differ substantially in emotional up-regulation of (conditioned) sexual arousal in general. Nevertheless, results discussed in chapter eight indeed suggest that men are more effective in emotional down-regulation of sexual arousal compared to women. It is quite intriguing that in women, the deployment of an emotional down-regulatory strategy even resulted in overall increased affective value and subjective sexual arousal towards both CSs. These results correspond to the findings of Both, Laan and Everaerd (2011), who studied the regulation of sexual arousal by means of attentional focus in healthy sexually functional men and women. In this study, women reported stronger absorption (i.e. the extent to which the participant experienced him or herself as a participant in the sexual activity shown in the film) in the cool attentional focus condition than in the no-instruction control condition, whereas men, as expected, reported lower absorption levels in the cool attentional focus condition than in the no-instruction control condition. Results from studies on negative emotion down-regulation (McRae et al., 2008) demonstrated that men have greater down-regulation of amygdala activity and less prefrontal activity during the regulation of negative affect, despite comparable levels of subjectively declared negative affective value in men and women. This suggests that men are able to generate and implement cognitive emotion down-regulation strategies with less effort or difficulty than women, at least in case of negative emotions. However, to the best of our knowledge, imaging studies on sexual emotion regulation in both sexes is lacking in the

literature, and as a consequence we can only speculate whether a similar mechanism accounts for the found results in this thesis. Besides, a pronounced difficulty in emotion down-regulation in women while processing sexual (conditioned) stimuli can also be the result of anatomical differences between men and women (Laan & Everaerd, 1995). Bodily responses and changes therein are an apparent aspect of emotional response. The association between genital and subjective sexual arousal is generally lower for women than for men (Chivers et al., 2004), possibly explained by the giving that men are likely to have more (visual and tactile) cues they can use to detect genital response than women do (Sakheim et al., 1984).

Given the problems in comparing genital responses of men and women directly, and possible differences between sexes with regard to responses to specific types of stimulus materials, it is far too early to infer that sex differences exist in basic sexual incentive learning, or to infer that women indeed use less efficient strategies in the down-regulation of positive (sexual) emotions than men

11.5. General Conclusions

First, although only few (well-controlled) studies have investigated classical conditioning of the sexual response in humans, results indicate that human sexual arousal can be conditioned. Second, results from chapter three indicate that DA receptor antagonism reduces sexual stimulation-induced genital sexual arousal, emphasizing the importance of DA availability in unconditional responding to sexual stimulation.

Third, results from chapters four and five raise doubt about the claim that, unlike other forms of classical conditioning, evaluative sexual conditioning is resistant to extinction. Unpaired presentations after (paired CS-US)

conditioning trials, reduce the magnitude of conditioned responses, including the magnitude of sexual evaluative learning effects. Hence, sexual evaluative learning is sensitive to extinction. Nevertheless, evaluative sexual learning effects are persistent, especially appetitive conditioned evaluative learning effects. Results suggest that extinction of sexual evaluative learning effects occurs at a slower rate than in other forms of associative learning (i.e. signal learning).

Fourth, chapter six provides evidence for the claim that an extinction procedure does not erase conditioned sexual associations in humans but instead involves new learning that is context dependent. Sexual extinction learning is especially dependent upon context. This makes clear that sexual arousal or the expectation of sexual reward can come under stimulus control by contextual cues associated with states of sexual reward.

Fifth, results from chapter seven suggest that administration of a single dose of DCS makes sexual extinction memories context-independent and prevents the return of conditioned sexual response in healthy sexually functional women. As a result, NMDA receptor glycine site agonists may be potential pharmacotherapies to reduce the motivational impact of sexual reward-associated cues, and to prevent relapse in sexual motivation disorders with a learned component.

Sixth, chapters eight and nine illustrate that (conditioned) sexual arousal can be modulated in line with participants' regulatory goals. In chapter eight, evidence was found for an emotional down-regulatory strategy to effectively enhance extinction of conditioned sexual responses in men. Results suggest that women seem to have some difficulty in the down-regulation of sexual arousal. Results from chapter nine suggest that the deployment of a cognitive up-regulatory strategy can efficiently increase unconditioned and conditioned sexual arousal elicited by sexual reward-conditioned cues in healthy men and women. Nevertheless, results are mixed on different response

modalities for men and women, making clear that the interpretation of the effectiveness of emotion regulation strategies is not straightforward.

Seventh, in chapter ten, making use of fMRI, it is demonstrated that regulation of sexual arousal depends on a neural circuit in which frontal cortical areas mediate the sexual responses generated at a subcortical level. Moreover, the results are in favour of the assumption that stress may complicate the successful cognitive down-regulation of sexual arousal, as evidenced by the increased neural activation in the right amygdala, right dorsal ACC, and right inferior frontal gyrus pars triangularis in the acute stress condition compared to the control condition during the down-regulation of sexual arousal. Results from this study indicate that acute-stress may be an important threat for the successful regulation of sexual arousal.

The research in this thesis indicate that lower level control processes, such as associative learning and related phenomena, as well as higher-level control process such as cognitive emotion regulation both function as control systems that regulate sexual arousal and behaviour. Returning to the model of sexual motivation and regulation discussed in the general introduction, the imbalance between (strong or weak) sexual urges and compromised cognitive control has been suggested to play an important role in the development of sexual motivation disorders. In this thesis, learning processes in sexual arousal response and cognitive control functions were investigated separately and in conjunction. This is of added value, since in case of disturbances in sexual arousal and motivation, it is especially important to understand how cognitive control processes function in the presence of sexual reward-related cues.

11.6. Limitations

Several comments are in order here. First, a limitation of the conditioning studies in this thesis is the absence of a between subjects (unpaired) control

group. Without such a control group it is difficult to determine whether and what learning has occurred. This makes it unclear if the observed conditioning effects are due to conditioning or to pseudo conditioning. Sensitization of sexual arousal would translate into increased genital and subjective responses across trails, and not in differential responding towards the CS+ and CS- per se.

Other limitations of these studies arise from the selected study cohort. The results were obtained in young healthy men and women. Moreover, results from the studies described in chapters three, and seven only encompassed healthy sexually functional women, whereas only men participated in the imaging study in chapter ten. Consequently, the applicability of the results to the general population has to be determined, and the generalization of the found results in chapters three, seven and ten to members of the opposite sex may not be straightforward. The generalization of our findings to other populations, such as adolescents, elderly individuals (<18 and >45 years), and clinical samples in particular, may also be hampered (see also section 11.7).

Moreover, in the current conditioning studies, vaginal photoplethysmography was used as indicator of physiological sexual arousal in women. Vaginal engorgement, however, is only one of many co-occurring processes during the sexual arousal response. Likewise, penile circumference was used as indicator of physiological sexual arousal in men. Like described in chapters six and eight, penile circumference in response to the CS+ was smaller as compared to penile responses towards the CS-. Although this phenomenon can be explained by physiological processes during the initial stage of penile erection, the measures that are used in the experimental conditioning studies (for men and women) relate to only some of many co-occurring processes, making them far from perfect. Therefore, additional methodology, such as thermal imaging or labial thermistor clips and equivalent penile thermistor (Payne & Binik, 2006), may provide additional insight in the physiological basis

for sexual arousal and human sexual learning. And as mentioned earlier, another caveat lies in the etiological relevance of the US. It is possible that for men (and possibly also for women), solely genital vibrostimulation is not the most effective sexual stimulus (Rowland & Slob, 1992; see also section 11.4. Sex Differences). Future studies on male sexual learning may consider vibrotactile stimulation combined with erotic film clips as US. Moreover, physiological sexual response is just one of the automatic measures to gauge on neurobiological mechanism involved in human sexual incentive learning. Functional imaging studies on sexual incentive learning, underlying neurochemical mechanisms, and related phenomena in both sexes is warranted to obtain complementary insight in neural mechanisms involved in sexual behaviours, which may help foster potentially critical insights in the aetiology of disorders in sexual motivation.

Regarding the promising effects of DCS in the aid of preventing renewal of maladaptive conditioned responses, only conclusions about the context-dependent recall of sexual extinction memory can be drawn. The unpaired US presentations at the beginning of each former acquisition context A on day two likely induced reinstatement effects mixed with the contextual renewal effects (Kalisch et al., 2006; Haaker et al 2013). Consequently, we are unable to differentiate between renewal and reinstatement effects on recall of sexual memory.

Another limitation of this thesis is that this thesis only addressed Pavlovian sexual conditioning, while this type of learning is not the only way in which humans may acquire certain sexual behaviours. For instance, individuals also learn about consequences of behaviours. In operant or instrumental learning associations are made between a behaviour and a consequence for that behaviour (Skinner, 1937; Thorndike (1911). From conditioning studies within other field of research, such as fear or anxiety it is known that operant learning

does not by default follow similar principles as seen in Pavlovian conditioning (Vurbic, Gold & Bouton, 2011).

With respect to the imaging study described in chapter ten, sexual arousal was not directly assessed: no ratings of subjective sexual arousal were obtained and no genital responses were measures. It can thus not be firmly concluded that the observed neural patterns in the down-regulation condition indeed resemblance decreased sexual arousal.

And lastly, and clinically relevant, the present study investigated only newly acquired sexual evaluative learning and relatively short-term effects within one (or two at most) experimental session.

11.7. Clinical Implications

As mentioned above, it is still to be elucidated whether the findings from the studies described in this thesis can be generalized to clinical practice. Nevertheless, hypotheses for future clinical work can be generated based on the present findings.

First, conditioned sexual likes and dislikes can be persistent, although conditioned affect eventually does extinguish (chapters four and five). This indicates that it might be beneficial to focus especially on subjective affect in the treatment of sexual arousal disorders with a learned component. A combination of extinction and counter-conditioning (learning a new opposite response) would plausibly be more effective than extinction alone in the treatment of these sexual disorders, especially in the treatment of sexual dislikes. In counterconditioning, the CS is paired with a stimulus evoking a response that is incompatible with the original unconditioned response, thereby altering the valence of a stimulus (Baeyens et al., 1992). However, present results make clear that depending on how strong and how easily available CS-US associations are, cue exposure therapy still seems relevant for the treatment

of sexual disorders with a learned component, like hypo or hypersexuality, since it is speculated that an extinction procedure makes the original CS-US associations less retrievable from memory, whereas it does enhance the accessibility of a new CS-no US association (Delamater, 2004).

In addition, the finding that sexual conditioned responses extinguish dependent upon context (chapter six) makes clear that extinction procedures may best be applied in the context in which the problematic behaviour is experienced, generalizing to other contexts and with multiple stimuli. Moreover, studies in clinical samples may transfer the effects of administration of a single dose of DCS on enhancing extinction memory, as shown in clinical trials with DCS (Price et al., 2013; Ressler et al. 2004; Santa Ana et al., 2009).

Moreover, in the treatment of problematic strong sexual arousal and appetite, cognitive strategies in the processing of conditioned sexual stimuli may be helpful. Learning to obtain effective emotion regulation strategies in circumstances in which sexual stimuli cannot be avoided may be useful to diminish undesirable feelings of sexual arousal and desire and to exert control over sexual behaviour. Therefore, future studies should incorporate clinical samples, like individuals with hypersexuality or deviant sexual preferences that manifest perturbed motivation. Likewise, in the treatment of problematic low sexual arousal and appetite, cognitive up-regulatory strategies of sexual arousal may be applied during initial conditioning stages in CBT in men and women. Results from chapter nine point to the utility of up-regulatory training for enhancing genital sexual arousal during the learning of new associations of sexually rewarding experiences and stimuli. In addition, emotion up-regulatory strategies may be promising add-on tools during therapeutic exercises in order to (re)create and enhance sexually pleasurable experiences. Therefore, future studies should assess the clinical efficacy of cognitive up- and down-regulatory strategies by including clinical samples, such as individuals with low sexual arousal and desire, or individuals with hypersexuality.

Lastly, the finding that acute stress seems to impair cognitive regulation of sexual arousal suggests that stress may be an important factor in the maintenance of disorders in sexual arousal and motivation. Although in our current study, only healthy sexually functional men were included, it could be hypothesized that chronic stress may eventually impair successful recruitment of structures implicated in top-down control, leading to a failure of regulation of sexual urges. Or individuals who frequently encounter sexual rewarding stimuli when under stress would run the risk of amplified incentive salience of sexual rewards and ultimately increased sexual motivation (Robinson & Berridge, 1993), which may be more difficult to control. Likewise, research has demonstrated that participants with low cortisol stress response demonstrate decreased NAc activation to (sexual) reward cues (Oei et al., 2014; Ossewaarde et al., 2011; Porcelli et al., 2012), which eventually may result in decreased sensitivity to sexual rewards and may possibly contribute to low sexual arousal and desire. However, more research is needed, especially in women and clinical samples. By investigating neural activation and (related) mental states, fMRI can make major contributions to the understanding of psychopathology and cognitive and affective networks in the brain. Moreover, fMRI also affords the opportunity to explore the feasibility of self-regulation of functional brain networks through neurofeedback. During fMRI neurofeedback training, participants receive feedback on their brain activity in real time and are instructed to change (i.e. up- or down-regulate) this activation (in line with the desired brain state) (Johnston et al., 2010). A better understanding of the neural changes accompanying successful regulation of sexual response may lead to the development of new treatment protocols targeting the functional correlates of specific brain networks. Moreover, since research has shown that individuals with high trait anxiety are impaired in regulating emotions (Indovina et al., 2011), probing the effects of stress–and the individual cortisol response- on

reward sensitivity might be helpful in predicting individual susceptibility to relapse.

11.8. Future Research Directions

As has become clear, there is a learning process underlying sexual arousal and behaviour and it involves forming associations between physical arousal and states of sexual reward with stimuli in the environment (i.e. incentives) (Toates, 2014). Animal research suggests, that once sexual preferences are established (Sisk & Foster, 2004), males are less susceptible to sexual learning to cues that differ too much from their developed preference (Coria-Avila, 2012; Pfaus, Kippin & Centeno, 2001). From research in rats it is known that at young age, the brain is sensitive to make new associations, including associations with sexual reward (Coria-Avial, 2012). Pfaus, Erickson and Talianakis (2013) suggest that an animal's initial sexual experiences are a sensitive or critical period during which neutral stimuli associated with sexual reward can become appetitive sexual CSs, whereas stimuli associated with sexual inhibition can become inhibitory CSs. Likewise, in humans, the first experience of (the absence of) sexual reward, especially ejaculation and orgasm, might have a similar strong effect in setting future preferences. The first experience of sexual arousal may be a powerful mediator of incentive formation and enhancement. Derived from the assumption that for many, adolescence and young adulthood represent a period of the first sexual experiences and sexual intercourse (Hawes et al., 2010), it can be speculated that especially this period in life is crucial for the development of sexual preferences and sexual behaviour (Pfaus et al., 2012). As the saying goes, 'neurons that fire together, wire together'. (Hebb, 1949). However, at present, very little is known about early sexual experiences in humans, and as a result it is unknown if a similar 'critical period' for the formation of sexual preferences does indeed exists in men and women.

Therefore, future studies should investigate if -similar to animals- humans also have an increased strength (presumably testosterone and DA based, see chapter two) of sexual incentive learning during adolescence and/or young adulthood. Likewise, the impact and strength of later sexual experiences on sexual preferences and sexual behaviour are also largely unknown. Investigating if sexual associations can be made more easily during a particular phase, or to investigate if such associations are weaker or more difficult to form during another (later) phase in life, is highly clinical relevant. CBT is based on associative learning principles and has emerged as the treatment of choice for disorders in sexual interest and desire (Both, S., Laan, & Schultz, 2010; Laan, & Both, 2008), including cue exposure therapy and behavioural techniques to (re)create different, more varied, or prolonged sexual stimulation to enhance sexually pleasurable experiences. In chapter six it was demonstrated that new learning during extinction inhibits, but does not erase, the CS–US association (Pavlov, 1927). Speculatively, a possible critical phase for sexual learning in humans may suggest that the original CS-US associations may be stronger, possibly due to the combination of high gonadal hormone and DA levels (Dominguez et al., 2001; Dominguez & Hull, 2001; Hermans et al., 2010 Wood, 2008), as compared to the later inhibitory CS–US associations or new CS-US associations that are made during the course of CBT. If there is indeed a difference in strength between the old (i.e. first sexual experiences) and new learned sexual associations that are made during CBT, pharmacological 'boosters' (e.g. DCS, or see Haaker et al., 2013) of new sexual associations or inhibitory CS–US associations may be a promising avenue to improving therapy for sexual motivation disorders.

In line with the above, another reason why future studies should focus on (sexual) experience, is the finding that experience seems to be a powerful buffer to devaluation. Prior exposure to sexually rewarding situations is thought to

vary considerably among subjects with sexual disorders, and insight into the influence of experience on conditioning may have a major impact on treatment resistance and 'relapse' risk. With the term devaluation, the decrease in conditioned response due to lowering the value of the unconditioned stimulus is meant (Bouton & Moody, 2004). Devaluation in Pavlovian conditioning may involve habituation to the solely US-presentations, after a CS-US acquisition phase. As a result of habituation, when the CS is presented again, the CS will elicit decreased CR. Interestingly, and in line with a theory of a 'critical period' in sexual learning (Pfaus, Kippin & Centeno, 2001), devaluation appears to be less strong when the acquisition of conditioned responding involves more CS-US parings (Bouton & Moody, 2004). Translating this to the clinical practice, an individual with a history of repetitively rewarding sexual experiences may be less susceptible to the intended disruptive effects of for instance anti-androgenic medication. Conversely, an individual with a limited history of sexual rewarding experiences may be more susceptible to disruptive effects of habituation in a long-term relationship. Indeed, results from fear research suggest that prior fear conditioning interferes with reward learning, subsequently leading to lower activation of the reward network (Bulganin, Bach & Wittmann, 2014). At present, human studies on devaluation in sexual conditioning are completely lacking, while they may provide highly relevant information for prevention of sexual disorders. Additionally, it would be of interest to investigate if individuals with sexual motivation disorders (e.g. women with Female Sexual Interest/Arousal Disorder, or men with hypersexuality and related disorders) have a decreased or increased susceptibility to incentive sexual conditioning as compared with healthy sexually functional individuals. Research on sexual learning in such clinical groups, combined with research on innate predispositions (i.e. genetic factor) may shed light on the neurodevelopmental trajectory of 'normal' and maladaptive sexual responses and behaviour. This knowledge about basic learning processes

involved in 'normal' and maladaptive sexual behaviours is crucial in the development of clinical treatments for those behaviours. Likewise, at present, the effect of counterconditioning on learned sexual evaluative effects in healthy participants but also in clinical samples is largely unknown (but see Davison, 1968 and Jackson, 1969), especially in case of low sexual arousal and desire. Counterconditioning in the treatment of paraphilia for instance, would consist of encouraging patients to visualize or imagine the targeted sexually-arousing stimulus while pairing this stimulus with an aversive stimulus (e.g. an aversive smell, a loud noise or a disgusting (mental image) until eventually the most sexually arousing image no longer yields sexual response, also at the evaluative level. Likewise, counterconditioning in the treatment of low sexual arousal would consist of (re)create different, more varied, or prolonged sexual stimulation to enhance sexually pleasurable experiences. These possible mechanisms in changing unwanted sexual CRs remain important directions for future research, including the neural mechanisms for appetitive-aversive interactions that are poorly understood, as it will likely yield important knowledge which may help in the development of clinical treatments for maladaptive sexual behaviours, including paraphilias and deviant sexual preferences that manifest perturbed motivation, but also for the more prevalent sexual desire and arousal disorders.

Moreover, no studies have been conducted on the role of DA in male sexual conditioning. Several factors, including sex chromosome complement (Lombardo et al., 2012), the presence of the sry gene (Dewing et al., 2006) and gonadal hormones, suggest that testosterone regulates incentive sensitivity through interactions with mesolimbic DA pathways (Hermans et al., 2010; Wood, 2008). This makes clear that future research on the role of DA in male sexual learning is warranted, as these findings may help in the understanding of the biological mechanisms underpinning addictive behaviours and how these

may affect vulnerability to drug abuse or the development of sexual dysfunctions in men. Additionally, since the mesolimbic DA system is not only crucially involved in appetitive motivational processes, but also in aversive motivational processes underlying learning and the execution of goal-directed behaviour (Robbins & Everitt, 1996; Robinson & Berridge, 2003), research on the role of DA in human aversive sexual learning is warranted. Especially, since results discussed in chapter five suggests that men and women seem to differ in sensitivity to aversive sexual conditioning. In the same sense, making use of imaging techniques, future studies in men and women, should investigate which neural circuits are involved in appetitive and aversive sexual learning and extinction, and in encoding of contextual information during extinction, and how these circuits can be modulated to further improve the effectiveness of extinction based therapies.

Next to DA, other neurotransmitter systems, including the opioid and serotonin systems, and gonadal hormones play an important role in sexual behaviour (Aubert et al., 2012; Holloway, 2012; Pfaus, Kippin & Centeno, 2001). Like mentioned in chapter two, it is suggested that testosterone regulates incentive sensitivity through interactions with mesolimbic DA pathways (Wood, 2008; Hermans et al., 2010), herewith creating a permissive environment that allows external sensory stimuli to induce DA release during sexual behaviour in animals (Dominguez et al., 2001; Dominguez & Hull, 2001). Although testosterone is known to affect sexual desire and arousal in humans (Hermans et al., 2010), the role of testosterone in human sexual incentive learning is largely unknown. Seen the importance of testosterone in regulating sexual incentive sensitivity, future research in humans is warranted.

In the distinction made by Berridge (2004), 'wanting' has been characterized as the value of incentive motivation held by a stimulus without any hedonic component, and is presumed to be mediated by DA functioning.

On contrast, 'liking' encompasses the hedonic aspect of a stimulus presentation, the positive sensory component that accompanies reward delivery and is thought to be mediated by the opioid system. As described in chapter two, there are two opioid systems. One is assumed to be related to incentive motivation, in which opioid systems in the VTA and the mesolimbic DA system are involved, and may relate to sexual motivation, and the other is thought to play a crucial role in the performance of certain behaviours involving endogenous opioids, like sexual performance (van Ree et al., 2000). In general, opioids and opioid drugs are found to have an inhibitory role in both male and female sexual behaviour (Pfaus & Gorzalka, 1987; Holloway, 2012). Administration of opioid antagonists (e.g. naloxone) in sexual conditioning experiments in animals seems to disrupt the incentive motivation for and/or hedonic value of a CS predicting sexual opportunity or of the sexual stimulus itself (Holloway, 2012). At present the role of the opioid system in human sexual incentive learning is unknown, despite the fact that insight in the role of this neurotransmitter system in human sexual motivation will bring innovative ideas, and is needed to guide psychological and/or pharmacological treatment for disorders in sexual motivation. In a similar manner, no studies on the role of serotonin in human sexual conditioning have been conducted. Research has shown that serotonin has sexual side effects such as decreased sexual desire (Meston & Frohlich, 2000), and serotonin reuptake inhibitors seem to have efficacy in treating hypersexuality (Bradford, 2001). Therefore, it would be worthwhile to examine how serotonin possibly inhibits the reward system activity during processing of sexual stimuli, and study the role of serotonin in associative sexual reward learning in general.

To return to Plato's reflection of human nature, the provocation of sexual appetite and cognitive control reflects sexual behaviour as a whole: the successful completion of the chariot´s journey towards enlightment and the truth is depending on the balance between emotions and man's appetites, ánd the cortical safeguards designed by evolution to control these sexual urges. Insights in the lower- and higher-level control processes may yield explanations, as to how learned maladaptive responses -as seen in hypersexuality or sexual interest/arousal disorder- may develop and how these problematic behaviours may be effectively treated.

Written ages ago, the Phaedrus myths had the primary function of raising questions about knowledge and truth while drawing attention to the limits of human capacities. The current journey towards the 'truth' as described in this thesis, aimed at contributing to the growing literature on learning mechanisms and cognitive regulation in sexual arousal, herewith adding to the foundation of our understanding of (neural) processes involved in sexual arousal, which hopefully may help in the development of cognitive behavioural and pharmacological treatment of disorders in sexual motivation. But as has become clear, research on sexual conditionability and sexual regulation, and related neuromodulatory systems in humans is in its infancy, meaning there are many topics that need further investigation. However, as Plato once stated: "*the beginning is the most important part of the work*".

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Nederlandse Samenvatting / Summary in Dutch

Achtergrond

Het begrijpen van seksuele opwinding en seksueel gedrag, en stoornissen hierin, vereist een theoretisch kader dat zowel de invloeden vanuit de biologie en cognitie omvat, alsook verenigbaar is met evolutionaire psychologie. Seksuele opwinding kan gezien worden als een evolutionair bewaarde emotie en wordt beïnvloed door lower-level en higher-level controlesystemen in het brein. Emotie kan gedefinieerd worden als een verandering in actiebereidheid, uitgelokt door bepaalde externe gebeurtenissen en door gedachten. Dit betekent dat emoties kunnen worden opgewekt door specifieke prikkels. In het geval van een seksuele emotie treden er specifieke genitale reacties op, de geslachtsdelen raken meer doorbloed. Daarnaast is te verwachten dat confrontatie met een seksuele stimulus ook leidt tot motorische preparatie; het brein stuurt signalen naar de spieren ter voorbereiding op seksuele actie. De actiegeneigdheid, die uitgelokt wordt door een seksuele stimulus, kan uiteindelijk uitmonden in daadwerkelijke seksuele activiteit. Ook heeft seksuele opwinding een subjectieve component: de ervaring van seksueel verlangen en seksuele opwinding.

Dit proefschrift richt zich op de vraag in hoeverre de menselijke seksuele opwindingsrespons onder invloed staat van lower-level controle processen zoals associatief leren en in welke mate higher-level controle processen zoals cognitieve emotieregulatie deze responsen kunnen beïnvloeden. Het eerste deel van dit proefschrift is geheel gewijd aan onderzoek naar klassieke conditionering van de seksuele opwindingsrespons. Dit eerste deel richt zich dus op lower-level controle processen in seksueel beloningsleren. Het tweede deel van dit proefschrift richt zich op de invloed van cognitieve emotieregulatie (dus higher-level processen) op seksueel beloningsleren en op

het verwerken van seksuele stimuli. Daarbij wordt ook gekeken naar de effecten van stress op de cognitieve down-regulatie van seksuele opwinding.

Incentive Motivatie Theorie en Seksueel Beloningsleren

Hoewel er stimuli zijn die van nature seksuele reacties en aangename gevoelens kunnen veroorzaken, zoals bijvoorbeeld het aanraken van de genitaliën, ontleent het merendeel van seksuele stimuli zijn betekenis aan leerprocessen: zowel positieve als negatieve seksuele associaties. Het potentieel van stimuli die seksueel verlangen en opwinding kunnen oproepen of kunnen afremmen is afhankelijk van de seksuele leergeschiedenis van het individu. Het wordt dan ook algemeen aangenomen dat associatief leren in de vorm van klassieke conditionering een rol speelt in de ontwikkeling van zowel 'gezond en normaal' seksueel gedrag, alsook in de ontwikkeling van stoornissen in seksuele motivatie, zoals bijvoorbeeld hyperseksualiteit, parafilieën, seksuele-interesse-/opwindingstoornis, of seksuele aversie. Aangezien associatieve leerprocessen een essentiële link vormen tussen stimulus en respons, en daarom aangrijpingspunt van interventie kunnen zijn in de behandeling van stoornissen in seksuele motivatie, is inzicht in hoe stimuli seksueel motiverende waarde kunnen verwerven of verliezen van groot belang.

Het is een gegeven dat mensen verschillen in hun behoefte aan seksuele activiteit, waarbij, gemiddeld genomen, mannen vaker zin lijken te hebben dan vrouwen. Hoewel dit geen probleem hoeft te zijn, kunnen grote discrepanties in behoeften of een sterk verminderd of te veel aan seksueel verlangen wel tot relationele problemen of persoonlijk lijden leiden. Geen zin in seks is bij vrouwen een veel voorkomende klacht in de seksuologische praktijk. Volgens de definitie van de Diagnostic and Statistical Manual of Mental Disorders (DSM-5) wordt seksuele-interesse-/opwindingstoornis bij de vrouw gekenmerkt door het ontbreken of een significante vermindering van seksuele belangstelling/opwinding. Bij verminderd seksueel verlangen speelt vooral het

ontbreken van zin in iets dat als positief beleefd kan worden een cruciale rol. Verminderd seksueel verlangen wordt om deze reden onderscheiden van seksuele aversie, aangezien bij seksuele aversie negatieve emoties (zoals walging of angst) een rol spelen. Echter, te veel zin in seks kan ook voor problemen zorgen, zeker wanneer dit zich ontwikkelt tot grensoverschrijdend seksueel gedrag. Hyperseksualiteit kan worden omschreven als een stoornis in de sterkte van het seksueel verlangen en kenmerkt zich door continu en herhaaldelijk bezig zijn met seks en uit zich in ongecontroleerd seksueel gedrag. Hyperseksualiteit heeft over het algemeen niet alleen negatieve gevolgen voor de persoon zelf, maar veelal ook voor de omgeving. Hyperseksualiteit betekent in het algemeen een verslechtering van het algemeen functioneren, en heeft bijvoorbeeld zijn weerslag op beroepsmatige activiteiten of heeft relationele problemen tot gevolg. Ook afwijkende seksuele voorkeuren (parafilieën), waarbij het seksueel verlangen en de seksuele interesse gericht zijn op een doel of onderwerp dat maatschappelijk niet aanvaard, of niet gangbaar is, kan persoonlijk lijden veroorzaken en het sociaal en beroepsmatig functioneren bemoeilijken.

De incentive-motivatie-theorie stelt dat seksuele motivatie niet zonder aanleiding ontstaat, maar dat deze altijd het gevolg is van interactie van organisme en omgeving. Dit model suggereert dat seksuele motivatie het resultaat is van een samenspel tussen de gevoeligheid van het seksuele responssysteem (beïnvloed door bijvoorbeeld hormonen en neurotransmitters in het brein) en stimuli die in de omgeving aanwezig zijn of imaginaire representaties hiervan. Dit betekent dat stimuli, en de betekenis hiervan, heel belangrijk zijn bij het ontstaan van seksuele motivatie. Stimuli in de omgeving of gedachten aan deze stimuli van een individu kunnen deze motivatie in gang zetten. Door middel van klassieke conditionering kunnen aanvankelijk (niet-seksuele) neutrale stimuli signalen worden voor seksuele beloning, waardoor ze motivationele waarde kunnen verkrijgen. Andersom, kunnen stimuli, welke

aanvankelijk positieve seksuele gevoelens opriepen, middels aversieve conditionering een negatieve motivationele waarde krijgen en zo hun aantrekkelijkheid verliezen. Een mogelijk voorbeeld hiervan is wanneer seks gepaard gaat met negatieve gevolgen zoals angst, teleurstelling of pijn bij het vrijen. Seksuele leermechanismen kunnen verklaard worden vanuit een evolutionair standpunt. Het is aannemelijk dat de ontwikkeling van deze leermechanismen reproductieve voordelen met zich mee brengt. Door middel van leren is de mens (maar zijn ook andere organismen) in staat om te reageren op- en zich aan te passen aan veranderingen in de omgeving, om gevaar te vermijden, en om toenadering te zoeken tot belonende situaties, waaronder seksueel belonende situaties. Er wordt verondersteld dat seksuele activiteit intrinsiek belonend of bekrachtigend is. Met name ejaculatie en orgasme kunnen gezien worden als primaire beloningen, en kunnen om deze reden leerprocessen bekrachtigen. Eén van de belangrijkste verklaringsmodellen voor het ontwikkelen en in stand blijven van stoornissen in seksuele motivatie die geen duidelijk medische oorzaak hebben, zoals hyperseksualiteit, parafilieën of seksuele-interesse-/opwindingstoornis bij de vrouw, is het concept van de klassieke of Pavloviaanse conditionering. Tijdens dit leerproces wordt een bepaalde neutrale stimulus, situatie of gebeurtenis (herhaaldelijk) geassocieerd met een seksueel belonende stimulus (bijvoorbeeld aanraking van de geslachtsdelen of orgasme; on-conditionele stimulus; unconditional stimulus, US). Er treedt associatief leren op, ook wel 'conditionering' genoemd, waarbij de van oorsprong neutrale stimulus een geconditioneerde stimulus (conditionele stimulus; conditional stimuls; CS) wordt. Door dit herhaaldelijk aanbieden van de US met de CS zal na enige tijd de respons die aanvankelijk enkel optrad na aanbieding van de US ook optreden na aanbieding van de CS (ook al wordt de US nu niet meer aangeboden). Deze respons op de CS wordt de geconditioneerde respons (geconditioneerde response; conditioned response, CR) genoemd. Het herhaaldelijk aanbieden van de CS zonder de US

zal echter na verloop van tijd resulteren in het uitdoven van de CR. Met andere woorden, deze CR zal extinctie laten zien.

Er kan beredeneerd worden dat bij personen die klachten rapporteren van weinig zin in seks, er wellicht een gebrek is aan associatie tussen seksueel belonende ervaringen en stimuli, met als gevolg dat maar een beperkt aantal stimuli seksueel verlangen kan oproepen. Daarentegen zijn er voor personen die aangeven veel behoefte aan seks te hebben, wellicht sterke en veelvuldige associaties geweest, met als gevolg dat een groot aantal prikkels seksueel verlangen kan oproepen.

Deel 1

Hoofdstuk twee: In dit hoofdstuk wordt een overzicht gegeven van studies - gepubliceerd voor oktober 2013- met dieren en mensen naar de rol van klassieke conditionering, leren en de rol van de neurotransmitter dopamine (DA) in seksuele opwinding en seksueel gedrag. In dit hoofdstuk worden de bevindingen in het kader van de incentive motivatie theorie beschreven. De beschreven onderzoeken met dieren wijzen uit dat klassieke conditioneringsprocessen een direct en robuust effect hebben op seksueel gedrag, zoals bijvoorbeeld te zien is in aangeleerde voorkeur voor een partner- of plaats. Bijvoorbeeld, onderzoek met ratten heeft aangetoond dat wanneer mannetjes ratten hun eerste ejaculatie krijgen tijdens geslachtsgemeenschap met vrouwtjes ratten die een bepaalde geur dragen (bijvoorbeeld amandel-geur), de mannetjes op een later testmoment een voorkeur hebben voor vrouwtjes ratten die naar deze geur ruiken in vergelijking met vrouwtjes ratten die geen geur dragen. De amandel-geur is aanvankelijk een neutrale stimulus, welke geen seksueel gedrag zal uitlokken bij de mannelijke ratten. Echter, na het koppelen van deze neutrale stimulus (amandel-geur) met de seksueel belonende stimulus

(ejaculatie; US), is deze geur een CS geworden. Confrontatie met de CS wordt direct geassocieerd met de seksueel belonende stimulus en roept een seksuele respons op. Op neurobiologisch niveau betekent dit dat er verbindingen ontstaan in het brein tussen verschillende representaties en dat zich geheugensporen vormen die bij de confrontatie met een CS worden geactiveerd. Niet alleen onderzoek met ratten, maar ook onderzoek bij vissen, kwartels, paarden en apen laat zien dat organismen op een dergelijke manier leren over seksueel belonende stimuli en situaties. Ook heeft onderzoek uitgewezen dat zowel ratten als kwartels een geconditioneerde voorkeur kunnen ontwikkelen voor een plaats of ruimte ('conditioned place preference') waarin eerder geslachtsgemeenschap heeft plaatsgevonden met een soortgenoot van het andere geslacht. Hierbij kunnen zowel visuele, auditieve, olfactorische of somatosensorische stimuli als CS dienen. Echter, hoewel arbitraire (bijvoorbeeld visuele of olfactorische) CSs effectief zijn gebleken in het uitlokken van een seksuele CR in mannelijke en vrouwelijke dieren, lijkt een grotere gelijkenis tussen CS-US garant te staan voor CRs die moeilijk uit te doven zijn. Oftewel deze CRs lijken resistent te zijn tegen extinctie. Dit effect werd voornamelijk bij mannelijke dieren waargenomen. Verder laten dierstudies zien dat verhoogde DA concentraties in beloningsstructuren in het brein, zoals de Nucleus Accumbens (NAc), een essentiële rol spelen bij beloningsleren, inclusief seksueel beloningsleren. Seksuele beloning (bijvoorbeeld ejaculatie, paced mating of vaginale/clitorale stimulatie bij ratten) blijkt een krachtige bemiddelaar van incentive formatie, en dit proces is afhankelijk van DA transmissie in het brein. Hoewel het onderzoek naar menselijk seksueel beloningsleren is achtergebleven ten opzichte van het onderzoek bij dieren, is gedegen onderzoek naar seksuele leerprocessen bij mensen de laatste jaren goed op gang gekomen. In het onderzoek bij mensen dient een erotische afbeelding of film vaak als US, maar ook vibraties welke worden toegediend aan de geslachtsdelen kunnen als US dienen. Daarbij is het bij mensen ook mogelijk

om de subjectieve beleving van seksuele opwinding vast te stellen door middel van vragen tijdens het onderzoek. Geconditioneerde responsen (CRs) kunnen gemeten worden door middel van objectieve procedures zoals fysiologische reacties. De fysiologische component van seksuele opwinding vertaalt zich bij vrouwen in veranderingen in vasocongestie (doorbloeding) van de vagina, en bij mannen in erectie van de penis. De genitale opwindingsrespons bij mannen kan gemeten worden met behulp van genitale meetinstrumenten die het volume of de omtrek van de penis meten, zoals bijvoorbeeld indium-gallium rekbandjes. De genitale opwindingsrespons bij vrouwen kan gemeten worden door middel van een vaginale fotoplethysmograaf. Dit meetinstrument (met de vorm van een tampon) detecteert met behulp van een sensor de toe- of afname in doorbloeding van de vaginawand en vertaalt dit in de zogenaamde vaginale puls amplitude (VPA). Het differentieel conditioneringsparadigma leent zich goed om seksuele conditionering te bestuderen, en het wordt om deze reden vaak toegepast. In een dergelijk paradigma worden er twee nagenoeg identieke stimuli als CSs gebruikt. Slechts één CS, de CS+, wordt gevolgd door US (bijvoorbeeld genitale vibratie) in de conditioneringsfase (of acquisitiefase), terwijl de andere CS, de CS-, nooit wordt gevolgd door de US. Tijdens deze conditioneringsfase leren de deelnemers dus de relatie tussen de CS+ en het krijgen van de US en wordt de CR verworven. Deze CR zal te zien zijn tijdens de hierop volgende extinctiefase, waarin de CS+ niet langer meer wordt gevolgd door de US. Aan het begin van deze extinctiefase zullen deelnemers namelijk een CR laten zien in reactie op de CS+ maar niet op de CS-. Echter, wanneer de CS+ herhaaldelijk wordt aangeboden zonder de US zal uitdoving (extinctie) van deze CR optreden. De eerste studies naar seksuele conditionering waren overwegend slecht gecontroleerd of betroffen case studies. De latere studies naar seksueel beloningsleren leveren aanwijzingen op dat de seksuele opwindingsrespons van mensen geconditioneerd kan worden. Ook zijn er aanwijzingen dat een grotere US-CS gelijkenis ook bij mannen

hierbij een rol speelt. Dit suggereert mogelijke het bestaan van 'prepared' associaties. Hoewel er tot op heden nog geen studies gedaan zijn naar de rol van DA bij seksueel beloningsleren bij mensen, zijn er wel aanwijzingen dat beloningsstructuren in het brein activatie laten zien tijdens seksuele conditionering. Op grond van deze onderzoeken kan dan ook geconcludeerd worden dat associatief beloningsleren een belangrijke rol speelt bij het tot stand komen van seksuele opwinding en seksueel gedrag,

Hoofstuk drie: Onderzoek heeft laten zien dat de neurotransmitter DA belangrijk is voor motivationele processen. DA is verantwoordelijk voor een plezierig effect en begeerte bij onder meer seks en drugs. Hoewel onderzoek bij dieren heeft laten zien dat DA transmissie essentieel is voor seksueel beloningsleren, ontbreekt experimenteel onderzoek hiernaar bij mannen en vrouwen in de onderzoeksliteratuur. In dit hoofdstuk wordt een studie beschreven naar het effect van het verlagen van DA spiegels middels een DA antagonist (DA 'blocker', haloperidol) op seksuele conditionering bij vrouwen. Het betrof een dubbelblind, placebo gecontroleerd experimenteel onderzoek, waarin deelneemsters random werden toegewezen aan een Placebo of aan een Haloperidol conditie. Een differentieel conditioneringsparadigma werd toegepast met twee neutrale afbeeldingen als CSs, en genitale vibrostimulatie als US. Slechts één van de CSs (de zogenoemde CS+) werd gevolgd door de US tijdens de acquisitiefase, terwijl de andere CS (de CS-) nimmer werd gevolgd door de US. VPA werd gemeten gedurende het hele experiment en subjectieve waarderingen na iedere CS aanbieding werden verzameld in de extinctiefase. Resultaten van dit onderzoek lieten zien dat de toediening van haloperidol invloed heeft op de ongeconditioneerde genitale opwindingsrespons bij vrouwen. Er werden geen aanwijzingen gevonden dat het verlagen van de DA spiegels van invloed is op de geconditioneerde seksuele respons (genitaal en subjectief). Dit kan echter ook verklaard worden door het feit dat de placebo conditie ook geen of een zeer zwakke geconditioneerde

seksuele respons lieten zien. Een duidelijke verklaring voor deze zwakke seksuele CRs in de placebo conditie hebben wij niet. Wel moet opgemerkt worden dat geconditioneerde seksuele responsen in een laboratorium setting altijd zeer klein zijn. Replicatie van dit onderzoek, ook onder mannen, is dus nodig om definitieve conclusies te kunnen trekken over wat de invloed is van haloperidol op seksuele conditionering.

Hoofdstuk 4: Een aangeleerde respons die eenmaal verworven is, is echter niet per definitie blijvend. Herhaaldelijke presentaties van de CS zonder de US zal leiden tot het uitdoven van de CR, aangezien de CS geen goede voorspeller meer is van de US. Dit leerproces is ook wel bekend als extinctie. Extinctie heeft klinische relevantie aangezien er wordt aangenomen dat dit het basismechanisme is van therapeutische behandelingen zoals cue-exposure therapie. Een mogelijke interventie bij de psychotherapeutische behandeling van stoornissen in seksuele motivatie, zoals hyperseksualiteit, kan inhouden om associaties die seksuele opwinding kunnen opwekken te verminderen of in kracht af te zwakken, met behulp van een extinctieprocedure. In cue-exposure therapie worden ongewenste aangeleerde responsen verminderd of afgezwakt door patiënten aan de cue (de CS) bloot te stellen zonder dat de US (bijvoorbeeld orgasme) hierop volgt. Uit onderzoek blijkt echter dat cue-exposure over het algemeen weinig doeltreffend is in het verhelpen van eenmaal verworven positieve (of negatieve) gevoelens. In klassieke conditionering lokt de CS een verwachting van de US uit en CR; het zogenaamde signaal leren (signal learning). Bij evaluatieve conditionering daarentegen, lokt de CS een automatische representatie van de US uit. Dit heeft tot gevolg dat evaluatieve leereffecten (geconditioneerde voor- en afkeuren) moeilijker te veranderen zijn door middel van extinctieprocedures. Geconditioneerde gevoelens van voor- en afkeur zijn dan ook hardnekkig. De resultaten van eerder onderzoek suggereerden dat ook op seksueel vlak eenmaal verworven positieve of negatieve gevoelens moeilijk zijn te verhelpen. Voor de

klinische praktijk is het van groot belang om te onderzoeken of en waarom deze geconditioneerde seksuele reacties zo hardnekkig zijn. In dit hoofdstuk wordt een studie beschreven waarin extinctie van appetitief geconditioneerde responsen werd onderzocht onder gezonde mannen en vrouwen. Twee neutrale afbeeldingen werden gebruikt als CS en slechts één CS (CS+) werd gevolgd door genitale vibratie (US). In de daaropvolgende extinctiefase werden de afbeeldingen bij herhaling (24x) in willekeurige volgorde aangeboden en de conditionele respons gemeten, bij mannen door de penis omtrek te meten, bij vrouwen door VPA te meten. Met behulp van vragen werd ook weer de emotionele waarde van de seksuele stimuli gemeten en de subjectief ervaren seksuele opwinding hierbij. Bij zowel mannen als vrouwen konden nu geen geconditioneerde genitale responsen worden waargenomen. Wel lieten de resultaten van deze studie zien dat geconditioneerde subjectieve seksuele opwinding en geconditioneerde affectieve evaluatie van de stimulus gedurende respectievelijk 20 en 24 extinctietrials geen complete uitdoving liet zien. Met andere woorden, ook al werd de CS+ gedurende 24 extinctietrials niet langer gevolgd door de genitale vibratie, mannen en vrouwen gaven 20 extinctietrials lang aan de CS+ seksueel opwindender te vinden dan de CS-, en zelfs gedurende 24 extinctietrials gaven zij aan deze CS+ als positiever te ervaren dan de CS-. Echter op de laatste extinctietrial was er geen verschil meer waarneembaar in deze positieve emotionele waarde jegens CS+ en CS-. Hoewel de appetitief geconditioneerde seksuele responsen dus niet geheel ongevoelig waren voor de extinctieprocedure (de CRs namen af in intensiteit gedurende de extinctietrials), suggereren deze resultaten dat extinctie niet de meest efficiënte behandeling is voor het verminderen van dergelijke aangeleerde responsen, aangezien er zeer veel extinctietrials nodig zijn willen deze subjectieve CRs uiteindelijk uitdoven. Appetitief aangeleerde seksuele responsen, zoals gezien in patiënten met hyperseksualiteit, kunnen wellicht beter worden behandeld door

een combinatie te maken van cue-exposure en interventies zoals counterconditioning of het toepassen van emotieregulatie technieken.

Hoofdstuk 5: In dit hoofdstuk wordt een studie beschreven waarin werd gekeken naar wat het effect is van een experimenteel toegediende aversieve stimulus op de seksuele respons. Er werd gebruik gemaakt van wederom een zeer uitgebreide extinctiefase (24 extinctietrials) om te onderzoeken of de geconditioneerde respons bij herhaalde expositie persisteert dan wel geringer wordt. Hiertoe werden twee erotische afbeeldingen als CS gebruikt en een pijnprikkel als US. In de conditioneringsfase werd slechts één CS (CS+) gevolgd door pijnlijke stimulatie aan de pols, terwijl de andere stimulus (CS-) nooit gevolgd werd door de pijnprikkel. In de daaropvolgende extinctiefase, werd de conditionele respons bij herhaling gemeten, bij mannen door de penis omtrek te meten, bij de vrouwen door VPA te meten. Met behulp van vragen werd ook de sterkte van positieve of negatieve gevoelens bij de seksuele stimuli gemeten en de subjectief ervaren seksuele opwinding. Resultaten van deze studie wezen uit dat de positieve emotionele waarde van een seksuele stimulus bij zowel mannen als vrouwen succesvol kan worden verminderd door deze stimulus te associëren met een pijnprikkel. Opvallend was echter dat alleen vrouwen aantoonbaar op fysiologisch niveau reageerden: zij lieten een lagere VPA zien in reactie op de CS+ (dus op de afbeelding die werd gekoppeld aan de pijnlijke prikkel) vergeleken met de CS-. Het verschil in positieve gevoelens in respons op de stimulus die eerder met de pijnprikkel werd aangeboden (CS+) en de stimulus die zonder pijnprikkel werd aangeboden (CS-) werd tijdens de extinctiefase zowel bij de mannen als de vrouwen geringer bij herhaling van de stimuli. Dit uitdoven van CRs gebeurde relatief snel, binnen slechts een aantal extinctietrials. Dit experimentele onderzoek leert ons dat aversief geconditioneerde seksuele voor- en afkeuren weliswaar de neiging hebben om te persisteren, maar uiteindelijk wel kunnen uitdoven. Cue-exposure therapie lijkt voor dergelijke aversief aangeleerde

ongewenste responsen (zoals verminderde gevoelens van seksuele opwinding en/of verminderde genitale opwinding tijdens het vrijen als gevolg van aversieve klassieke conditionering) een passende en efficiënte interventie. Door regelmatig pijn te ervaren tijdens het vrijen kunnen seksuele prikkels door de associatie met pijn een negatieve betekenis krijgen en minder seksuele opwinding oproepen. In de klinische praktijk zien we dit terug, pijn bij het vrijen (dyspareunie) gaat vaak samen met minder zin in seks en met opwindingsproblemen. De resultaten van de hierboven beschreven studie suggereren dat wanneer je de (pijnlijke) US wegneemt (dus geen dingen meer doen die pijn doen tijdens seks) seksuele prikkels weer hun (positievere) betekenis kunnen herwinnen en zo seksuele opwinding weer kan terugkeren.

Hoofdstuk 6: Uit onderzoek is bekend dat het aanbieden van CS presentaties niet resulteert in het geheel 'wissen' van de oorspronkelijk aangeleerde CS-US associatie. Onderzoek heeft aangetoond dat deze associatie bewaard blijft. Geconditioneerde responsen kunnen namelijk terugkomen (renewal) als gevolg van een verandering in context. Renewal is het herstel van CR in context A, maar niet in context B, wanneer verwerving (acquisitie of conditionering) van de CR heeft plaatsgevonden in context A en extinctie heeft plaatsgevonden in context B. In de klinische praktijk kan het renewal fenomeen herkend worden in terugval (relapse; klachten treden weer op) bij patiënten na het verlaten van de behandelsetting. Hoewel het renewal fenomeen, en de essentiële rol van context daarbij, uitvoerig is onderzocht onder dieren en bij angst, is dergelijk onderzoek bij appetitief seksueel leren onder mensen schaars. In dit hoofdstuk wordt een studie beschreven naar de invloed van context op het terugkeren (renewal) van geconditioneerde seksuele responsen bij gezonde seksueel functionele mannen en vrouwen. Twee neutrale afbeeldingen werden als CS gebruikt en slechts één CS (CS+) werd gevolgd door genitale vibratie tijdens de conditioneringsfase. Na de conditioneringsfase volgde een extinctiefase, en hierna volgde een testfase. Middels verschillende kleuren tl-

licht (geel en paars) werd de context van de experimentele ruimte gemanipuleerd. Deelnemers werden random toegewezen aan een AAA of ABA conditie. Deelnemers in de AAA conditie ontvingen conditionering, extinctie en de testfase in één context: context A (als verlichting van de experimentele ruimte werd slechts één kleur tl-licht gebruikt; geel of paars). Bij deelnemers in de ABA conditie vond de conditioneringsfase plaats in context A (bijvoorbeeld gele tl-verlichting), de extinctiefase in context B (paarse tl-verlichting) en de testfase weer in de originele conditioneringscontext A (gele tl-verlichting). Gedurende het gehele onderzoek werd bij mannen de omtrek van de penis gemeten, en bij vrouwen VPA. Met behulp van vragen werd ook weer de emotionele waarde van de seksuele stimuli gemeten en de subjectief ervaren seksuele opwinding daarbij. Ook werd door middel van vragen vastgesteld in welke mate de deelnemers de US verwachtten na het zien van de CSs (US expectancy). Zoals verwacht lieten deelnemers in zowel de AAA als ABA conditie geconditioneerde seksuele responsen zien, en deze responsen lieten uitdoving zien gedurende de extinctiefase. Tijdens de testfase lieten deelnemers in de AAA conditie geen renewal van seksuele CRs zien. In lijn met de verwachtingen lieten deelnemers in de ABA conditie echter wel renewal van geconditioneerd responderen zien wanneer zij weer de originele conditionering (acquisitie) context gepresteerd kregen na de extinctiefase. Deze resultaten laten zien dat uitdoving en het terugkeren van seksuele responsen in mensen context-afhankelijk is. Ook laten deze resultaten zien dat een extinctieprocedure seksuele associaties niet geheel uitwist, maar dat extinctie-leren een andere vorm van leren is, welke context afhankelijk is. Dit suggereert dat op dergelijke manier aangeleerde seksuele responsen niet uitgewist kunnen worden met behulp van cue-exposure interventies (tijdens dergelijke interventies worden inhibitoire associaties aangeleerd), en dat deze ongewenste seksuele responsen terug kunnen komen afhankelijk van context. Het verdient dan ook aanbeveling om ongewenste seksuele responsen te behandelen met cue-exposure in de

omgeving waarin dit problematische gedrag ervaren wordt en niet enkel binnen een behandelsetting. Het is echter onmogelijk om op een dergelijke manier in alle mogelijke situaties en contexten deze interventie toe te passen. Hierdoor zal er waarschijnlijk altijd een risico op terugval blijven voor patiënten wanneer zij met een bepaalde stimulus of situatie geconfronteerd worden. Processen die het extinctiegeheugen kunnen versterken en de contextafhankelijkheid van extinctieleren kunnen verminderen lijken dan ook veelbelovend. Een voorbeeld hiervan is bijvoorbeeld beïnvloeding van de N-methyl-D-aspartaat of NMDA-receptor, één van de receptoren van het glutamaat-systeem die een belangrijke rol speelt bij leerprocessen, met name door de stof D-cycloserine (DCS).

Hoofdstuk 7: NMDA-receptoren zijn een bijzonder type glutamaat-receptoren en komen op verschillende plaatsen in het brein voor. Onderzoek heeft laten zien dat NMDA-receptoren een belangrijke rol spelen bij leerprocessen en bij de vorming van 'geheugensporen' (ook wel lange termijn potentiatie, long-term potentiation, LTP genoemd). Studies naar farmacologische interventies in associatief leren (klassieke conditionering) en uitdoving van deze aangeleerde responsen (het zogenaamde extinctie leren) op seksueel vlak zijn helaas schaars in de wetenschappelijke literatuur. Een zeer recent onderzoek bij ratten naar de werking van D-cycloserine (DCS, een partiële NMDA-receptor agonist) op extinctiegeheugen heeft echter laten zien dat de toediening van DCS appetitief extinctiegeheugen versterkt en dit context-onafhankelijk maakt. In het experiment dat wordt beschreven in dit hoofdstuk werd seksuele conditionering en extinctie, in combinatie met het toedienen van een NMDA-antagonist (DCS) onderzocht bij gezonde vrouwen. Er werd verwacht dat toediening van DCS na een extinctiefase op dag 1 de consolidatie van seksueel extinctiegeheugen zou versterken, vergeleken met het innemen van placebo. Deelnemers worden random toegewezen aan een van de twee condities. Het betrof een dubbelblind, placebo gecontroleerd experimenteel onderzoek. Een differentieel conditionering-paradigma werd

toegepast, waarin twee stimuli werden gepresenteerd, waarvan er slechts één (CS+) gekoppeld werd aan een seksuele ongeconditioneerde stimulus (US). Als seksuele US werd vibrotactiele stimulatie van de genitalia toegepast. Twee gelijke (nagenoeg identieke) foto's van de onderbuik van een persoon van het andere geslacht dan de deelnemer fungeerde als geconditioneerde stimuli (CSs; de foto's verschillen in kleur (Blauw of Geel)). Welke CS (Geel of Blauw) als CS+ diende (en werd gevolgd door de genitale vibrostimulatie tijdens de acquisitiefase) werd gecounterbalanced. Context tijdens de acquisitie en extinctiefasen werd gemanipuleerd door de lichtomstandigheden (middels paars of geel licht) te veranderen in de experimentele ruimte. Het onderzoek had plaats op twee opeenvolgende dagen. Op dag 1 werd gedurende de acquisitiefase (A1) de CS+ 10 keer aangeboden en direct gevolgd door de US (vibrotactiele stimulatie). De acquisitiefase vond plaats in context A (lichtkleur A: random toegewezen kleur licht, geel of paars). De daarop volgende extinctiefase (B1) vond plaats in context B (vanzelfsprekend de andere kleur dan werd gebruikt om context A te creëren). Gedurende de extinctiefase werden de CS+ en de CS- ieder 10x aangeboden. De CS+ werd nu niet meer gevolgd door de US. Na de extinctiefase volgde direct een nieuwe acquisitiefase (A2) in context A, en een nieuwe extinctiefase (B2) in context B. Gedurende de pre-conditioneringsfase, de acquisitiefasen en de extinctiefasen werd genitale seksuele opwinding vastgesteld door middel van een vaginale fotoplethysmograaf, en werden ratings verzameld over de mate van verwachting van het krijgen van vibratie (US Expectancy), de emotionele valentie en de subjectieve seksuele opwinding. Direct na de tweede extinctiefase (B2) op dag 1 kregen de proefpersonen of DCS of placebo toegediend. Na 24 uur volgde een testfase, welke bestond uit presentaties van de CS+ en CS- in beide contexten, waarbij wederom de genitale seksuele opwinding werd vastgesteld en ratings van mate van verwachting van vibratie, van emotionele valentie en subjectieve seksuele opwinding werden verzameld. De resultaten

wezen uit dat vergeleken met de Placebo conditie, de DCS conditie zwakkere of geen genitale en subjectieve seksuele CRs liet zien op het testmoment 24 uur later, los van de context waarin op dag 1 extinctie-leren plaats vond. Het toedienen van DCS na extinctie-leren faciliteert dus de consolidatie van het seksuele extinctie geheugen en maakt dit context-onafhankelijk en voorkomt zo het terugkeren (renewal) van geconditioneerde seksuele responsen. Deze resultaten suggereren dat NMDA receptor glycine agonisten, zoals DCS, mogelijk veelbelovende farmacotherapieën kunnen zijn voor de preventie van terugval (relapse) bij stoornissen in seksuele motivatie met een leercomponent. Echter moet worden aangemerkt dat replicatie van dit onderzoek nodig is, ook onder mannen, voordat hier echt definitieve uitspraken over kunnen worden gedaan.

Deel 2

Mensen zijn in staat om invloed uit te oefenen op de emoties die ze ervaren en op de manier waarop emoties worden uitgedrukt. Dit kan ook wel omschreven worden door de term 'emotieregulatie' die verwijst naar processen die verantwoordelijk zijn voor het controleren, evalueren en bijstellen van emotionele reacties. Met cognitieve emotieregulatie staan de bewuste cognitieve processen van omgaan met emotie opwekkende informatie centraal. Voorbeelden hiervan zijn bijvoorbeeld 'reappraisal' (oftewel herwaarderen, anders interpreteren) of 'attentional deployment' (het intensiveren van de aandacht, of juist afleiden van de aandacht op een bepaalde emotie). Deze cognitieve regulatie processen zijn voorbeelden van higher-level controleprocessen. Door verschillen in de manier waarin individuen hun emoties reguleren wordt in het algemeen verondersteld dat cognitieve emotieregulatie een belangrijke factor speelt in de ontwikkeling van psychopathologie en wellicht ook in de ontwikkeling van stoornissen in

seksuele motivatie. Het falen van top-down controlemechanismen over bottom-up processen (zoals seksueel beloningsleren) speelt hierbij mogelijk een rol. Ondanks de veronderstelling dat inzicht in deze mechanismen, inclusief de interactie van deze lower-level en higher-level controlemechanismen, van belang is voor de ontwikkeling van effectieve behandelingen voor stoornissen in seksueel verlangen, ontbreekt onderzoek naar de invloed van cognitieve emotieregulatie op de verwachting van seksuele beloning in de literatuur.

Hoofdstuk acht: In dit hoofdstuk wordt een studie beschreven naar de invloed van het toepassen van een cognitieve emotie down-regulatie techniek, in de vorm van attentional deployment, op seksueel beloningsleren bij gezonde mannen en vrouwen. In deze studie werd een differentieel conditionering-paradigma toegepast, waarin twee stimuli werden gepresenteerd, waarvan er slechts één (CS+) gekoppeld werd aan een seksuele ongeconditioneerde stimulus (US). Als seksuele US werd vibrotactiele stimulatie van de genitalia toegepast. Twee gelijke (nagenoeg identieke) foto's van de onderbuik van een persoon van het andere geslacht dan de deelnemer fungeerde als geconditioneerde stimuli (CSs; de foto's verschillen in kleur (Blauw of Geel)). Welke CS (Geel of Blauw) als CS+ diende (en werd gevolgd door de genitale vibrostimulatie tijdens de acquisitiefase) werd gecounterbalanced. Deelnemers werden willekeurig toegewezen aan een '*Attend*' (controle) of een '*Down-reguleer*' conditie. Deelnemers in de Attend conditie kregen de cue 'attend' (opletten) gepresenteerd voor het zien van de CSs in de acquisitie- en extinctie fasen, terwijl deelnemers in de Down-reguleer conditie de cue 'down-reguleer' gepresenteerd kregen voor het zien van de CSs in de acquisitie- en extinctie fasen. Deelnemers werden vooraf aan het experiment geïnstrueerd dat zij bij het zien van de cue 'attend' enkel goed op dienden te letten, terwijl zij bij het zien van de cue "Down-reguleer' de emotionele seksuele impact van de CS+ dienden te verminderen door op de kleur van de CS te letten (en hierbij een geruststellend beeld uit de natuur met de kleur geel

of blauw voor de geest moesten halen). Het toepassen van een emotie down-regulatie strategie versterkte extinctie van geconditioneerd seksueel responderen bij mannen. Bij vrouwen resulteerde het toepassen van een dergelijke strategie in verminderde geconditioneerde toenaderingsneigingen jegens de CS+. Deze resultaten suggereren dat top-down processen (oftewel cognitieve higher-level processen) geconditioneerde seksuele responsen kunnen beïnvloeden.

Hoofdstuk negen: In dit hoofdstuk wordt de invloed van een cognitieve up-regulatie techniek op seksueel beloningsleren onderzocht bij gezonde mannen en vrouwen. Het experiment was nagenoeg hetzelfde als beschreven in hoofdstuk acht, echter werd nu een '*Attend*' conditie vergeleken met een '*Up-regulatie*' conditie. Deelnemers werden nu geïnstrueerd om bij het zien van de cue 'vergroot' de seksuele opwinding en seksuele responsen te versterken die door presentatie van de CS+ werden uitgelokt. Resultaten van deze studie lieten zien dat seksuele opwinding kon worden versterkt door het toepassen van de emotieregulatie strategie. Deze resultaten van hoofdstuk acht en negen suggereren dat emotieregulatie technieken wellicht behulpzaam kunnen zijn bij de behandeling van seksuele motivatie stoornissen met een leercomponent.

Hoofdstuk tien: Ondanks de veronderstelling dat een verstoorde emotieregulatie en een gevoeligheid voor stress mogelijk een rol spelen in de ontwikkeling van problematisch seksueel gedrag, is er weinig bekend over de interactie van deze factoren. In dit hoofdstuk wordt een functionele magnetische resonantie imaging (fMRI) studie beschreven naar het effect van het toepassen van een emotie down-regulatie strategie tijdens het verwerken van seksuele stimuli, alsook het effect van stress hierop. Er werd verondersteld dat activatie in beloningscentra in het brein (zoals de NAc en amygdala) zou afnemen in overeenstemming met emotionele down-regulatie, terwijl activatie in prefrontale structuren juist zou toenemen. Hierbij werd verondersteld dat stress (geïnduceerd middels de Trier Social Stress Test; TSST) de mogelijkheid

tot het down-reguleren van seksuele opwinding mogelijk zou bemoeilijken. Mannelijke deelnemers werden willekeurig toegewezen aan een 'Stress' of 'Controle' conditie en werden geïnstrueerd om hun seksuele opwinding in reactie op de seksuele stimuli te 'versterken' (Seks-Up), 'gelijk te houden' (Seks-Gelijk) of 'af te zwakken' (Seks-Down). Blood Oxygen Level Dependent (BOLD) responsen in reactie op de seksuele stimuli werden vergeleken met BOLD responsen in reactie op neutrale stimuli (Neutral), en ook werd de subjectieve waardering van het succes van regulatie verzameld. Deelnemers in beide condities lieten tijdens het down-reguleren van seksuele opwinding een toegenomen activiteit in prefrontale gebieden zien. Deelnemers in de stress conditie lieten een verhoogde activiteit zien in de rechter amygdala, rechter dorsale anterior cingulate cortex (ACC) en in de rechter inferior frontal gyrus pars triangularis (IFG) wanneer Seks-Down met Seks-Gelijk trials werden vergeleken. Deze resultaten suggereren dat stress mogelijk de cognitieve down-regulatie van seksuele opwinding kan aantasten.

Conclusie: Concluderend wijzen de resultaten uit dit proefschrift erop dat de menselijke seksuele respons vatbaar is voor lower-level en higher-level controleprocessen. Onderzoek laat zien dat de seksuele respons klassiek geconditioneerd kan worden en experimenteel onderzoek zoals beschreven in dit proefschrift, heeft laten zien dat deze geconditioneerde responsen onderworpen zijn aan een aantal wetten (conditioneringswetten), zoals extinctie en renewal. Ook heeft onderzoek in dit proefschrift aangetoond dat seksueel geconditioneerde evaluatieve leereffecten niet geheel ongevoelig zijn voor een extinctie procedure. Echter, de appetitief seksueel geconditioneerde evaluatieve leereffecten lijken hardnekkiger te zijn dan de aversief seksueel geconditioneerde evaluatieve leereffecten. Daarnaast laat onderzoek in dit proefschrift zien dat seksueel extinctie leren context afhankelijk is, maar dat DCS dit seksueel extinctie geheugen context onafhankelijk lijkt te maken. NMDA receptor agonisten kunnen om deze reden wellicht veelbelovend zijn

bij de behandeling van stoornissen in seksuele motivatie met een leercomponent. Er is echter meer onderzoek nodig, onder mannen en onder klinische groepen, om hier definitieve uitspraken over te kunnen doen. Ook laat dit proefschrift zien dat gezonde proefpersonen in staat zijn om middels het toepassen van cognitieve strategieën invloed uit te oefenen op de seksuele respons tijdens seksueel beloningsleren. Dit suggereert dat emotieregulatie-technieken wellicht behulpzaam kunnen zijn bij de behandeling van seksuele motivatie stoornissen met een leercomponent. Tot slot suggereren resultaten uit dit proefschrift dat stress het kunnen beheersen en afzwakken van seksuele opwinding mogelijk kan bemoeilijken.

Bij het interpreteren van de resultaten in dit proefschrift moet rekening gehouden worden met het feit dat alle experimentele studies zijn uitgevoerd onder gezonde seksueel functionerende proefpersonen. Dit maakt generalisatie van de onderzoeksbevindingen naar andere populaties lastig tot onmogelijk. Toekomstig onderzoek is dus nodig om te onderzoeken of de beschreven bevindingen ook buiten de in dit proefschrift onderzochte populaties bewaarheid kunnen worden.

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Curriculum Vitae

Mirte Brom was born on the 28th of May 1983 in Amstelveen, The Netherlands. In 2001 she obtained her VWO (high school) diploma at the Keizer Karel College in Amstelveen, The Netherlands. She studied Psychology at the University of Amsterdam and in 2011 she received her Master's degree (*Cum Laude*) in Clinical Neuropsychology. From 2011-2015 she worked on the PhD-project 'Memory for Sexual Reward' at Leiden University/ Leiden University Medical Centre. This thesis is the result of her work during that period. Mirte currently continues to work at the Leiden University Medical Centre as a Postdoctoral Researcher.