The role of incentive learning and cognitive regulation in sexual arousal

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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.

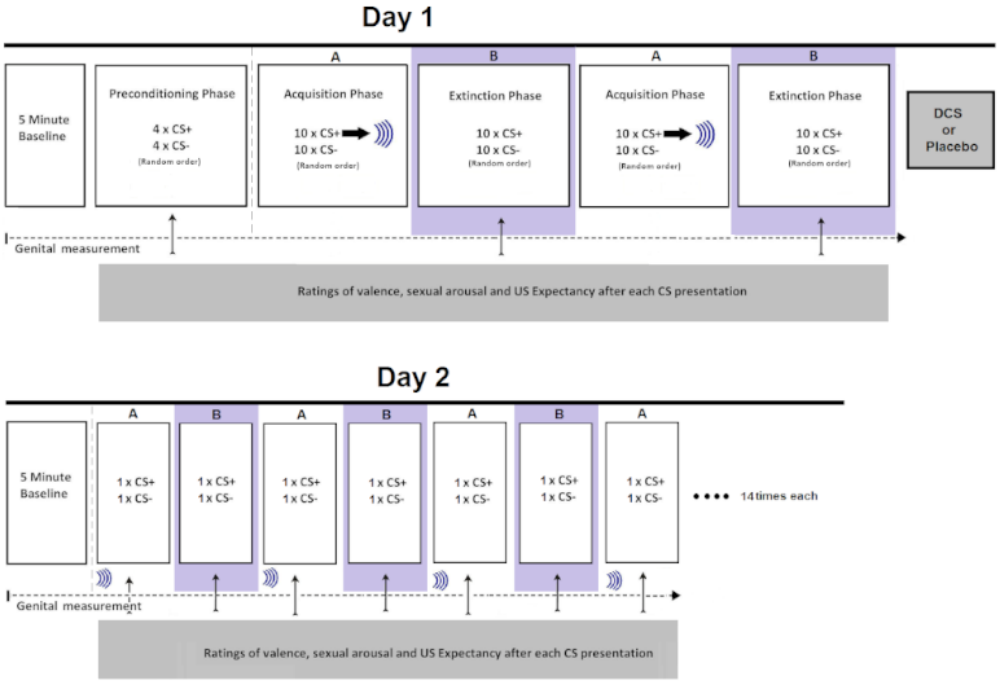

Figure 1. Schematic overview of the experimental procedure. **Day 1:** In the preconditioning phase, participants saw four (nonreinforced) presentations of each CS. In the acquisition phase in context A, the CS+ and CS- were presented 10 times each and 8 out of 10 CS+ presentations were followed by the 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.

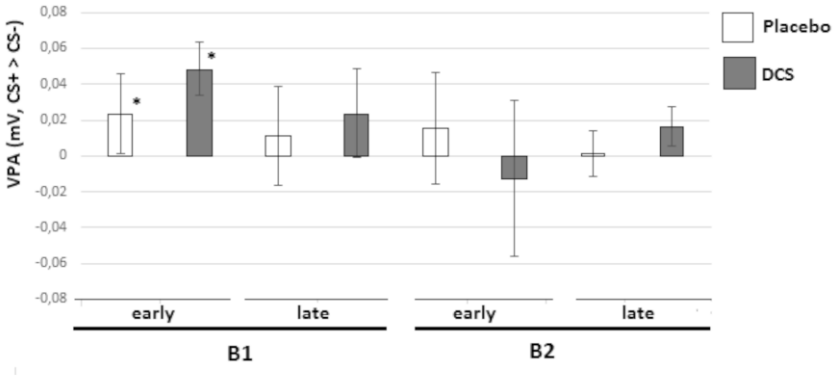

Figure 2. Vaginal Pulse Amplitude (VPA) Day 1. Mean Vaginal Pulse Amplitude (VPA) change scores from baseline (±S.E.M.) towards the CS + and CS- for the first 5 extinction trials (early), and for the last 5 extinction trials (late) in the first extinction (B1) phase and second extinction phase (B2).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

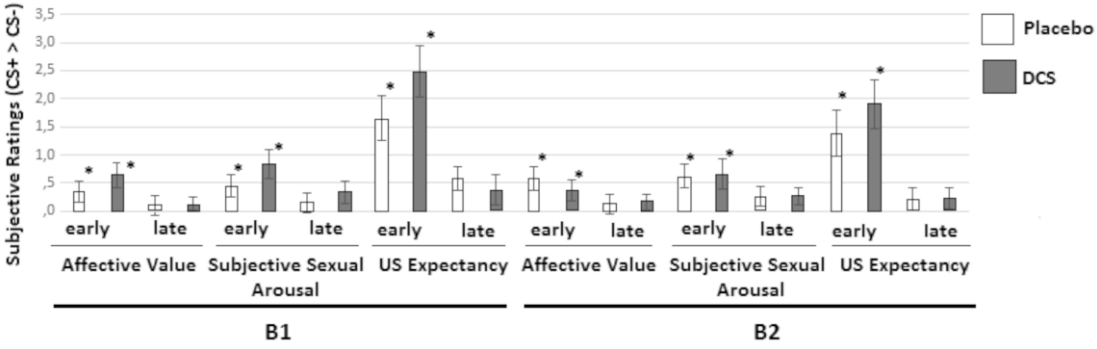

Figure 3. Subjective measures Day 1. Subjective measures CS+ > CS- scores (±S.E.M.) for the first (early) and last (late) extinction trial in the first extinction (B1) phase and second extinction phase (B2), for the placebo and DCS condition. *****significant differential responding towards CS+ and CS-.

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.

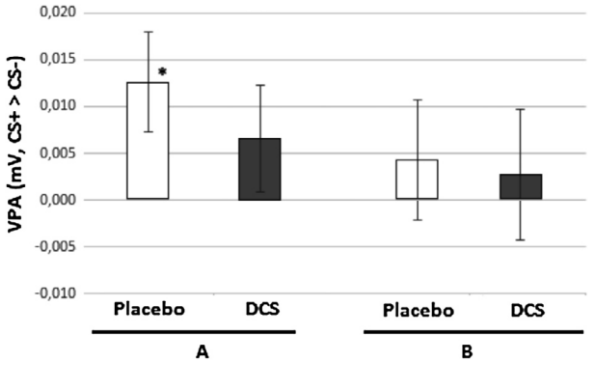

Figure 4. Mean VPA (Vaginal Pulse Amplitude) difference CS+ > CS- (±S.E.M.) on day 2 in the original acquisition context A, and in the extinction context B for the Placebo and DCS condition. *****Only participants in the Placebo condition demonstrated significant differential responding towards CS+ and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

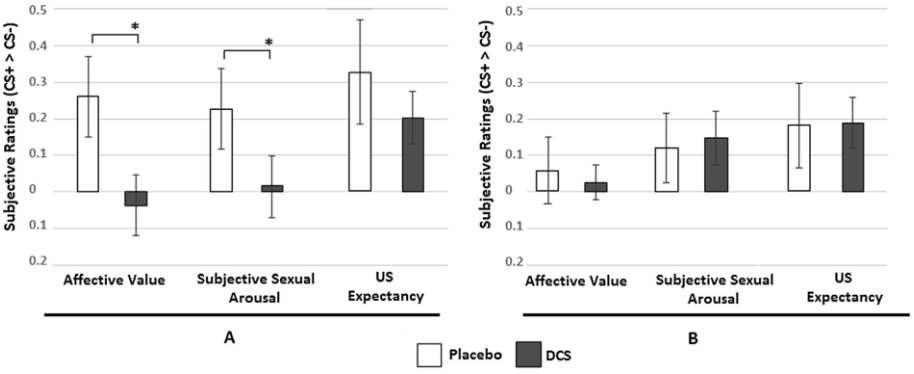

Figure 5. Effects of post learning DCS on subjective correlates (ratings of US Expectancy, Affective Value and Subjective Sexual Arousal; difference CS+ > CS-, and ±S.E.M) of recall of sexual extinction memory on day 2 in the original acquisition context A (left), and in the extinction context B (right).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

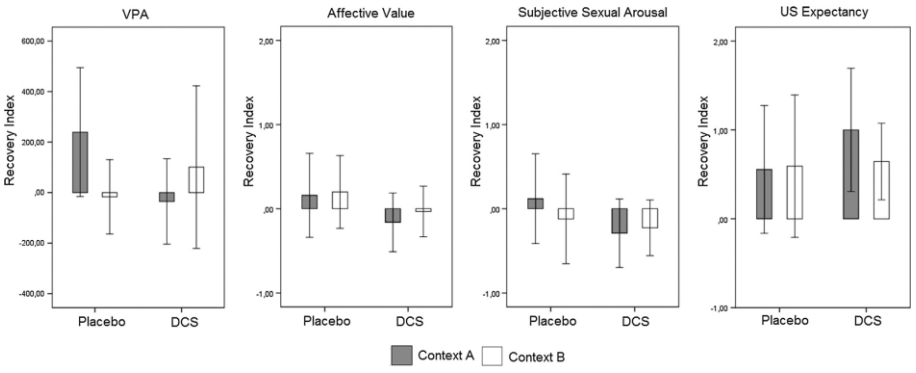

Figure 6. Recovery index: recovery in CR in the DCS and Placebo conditions (first trial day 2 minus last extinction trial day 1) for CS+ > CS− and ±S.E.M.

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.

References

Bechara, A., Tranel, D., Damasio, H., Adolphs, R., Rockland, C., & Damasio, A.R. (1995). Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science*, 269, 1115-1118.

Bouton, M.E. (2004). Context and behavioral processes in extinction. *Learning & Memory*, 11, 485–494.

Bouton, M.E., Vurbic, D., & Woods, A.M. (2008). D-cycloserine facilitates context-specific fear extinction learning. *Neurobiology of Learning and Memory*, 90, 504-510.

Brom, M., Both, S., Laan, E., Everaerd, W., & Spinhoven, P. (2014a). The role of conditioning, learning and dopamine in sexual behavior: A narrative review of animal and human studies. *Neuroscience & Biobehavioral Reviews*, 38, 38-59.

Brom, M., Laan, E., Everaerd, W., Spinhoven, P., & Both, S. (2014b). Extinction and renewal of conditioned sexual responses. *PloSONE*, e105955.

Brom, M., Laan, E., Everaerd, W., Spinhoven, P., Cousijn, J, & Both, S. (2015). The influence of Emotion Down-Regulation on the Expectation of Sexual Reward. *Behavior Therapy* 46, 379-394.

Carter, R. M., O'Doherty, J. P., Seymour, B., Koch, C., & Dolan, R. J. (2006). Contingency awareness in human aversive conditioning involves the middle frontal gyrus. *NeuroImage*, 29, 1007-1012.

Cohen, J. (1988). Statistical Power Analysis for the Behavioral Sciences (2nd ed.). New Jersey: Lawrence Erlbaum.

Delamater, A. R., & Westbrook, R. F. (2014). Psychological and neural mechanisms of experimental extinction: a selective review. *Neurobiology of Learning and Memory* 108, 38–51.

Di Chiara, G. (1999). Drug addiction as dopamine-dependent associative learning disorder. *European Journal of Pharmacology* 375, 13–30.

Fitzgerald, P.J., Seemann, J. R., & Maren, S. (2014). Can fear extinction be enhanced? A review of pharmacological and behavioral findings. *Brain Research Bulletin*, 105, 46–60.

Ghashghaei HT, Hilgetag CC, Barbas H. (2007). Sequence of information processing for emotions based on the anatomic dialogue between prefrontal cortex and amygdala. *Neuroimage* 34, 905–23.

Grillon, C. (2009). D-Cycloserine facilitation of fear extinction and exposure-based therapy might rely on lower-level, automatic mechanisms. *Biological Psychiatry*, 66, 636-641.

Haaker, J., Gaburro, S., Sah, A., Gartmann, N., Lonsdorf, T. B., Meier, K., Singewald, N., Pape, H.-C., Morellini, F., & Kalisch, R. (2013). Single dose of L-dopa makes extinction memories context-independent and prevents the return of fear. *PNAS*, 110, E2428–E2436, doi: 10.1073/pnas.1303061110

Haaker, J., Golkar, A, Hermans, D & Lonsdorf, T. B. (2014). A review on human reinstatement studies: an overview and methodological challenges. *Learning & Memory*, 21, 424-440.

Hoffmann, H., Janssen, E., Turner, S.L. (2004). Classical conditioning of sexual arousal in women and men: effects of varying awareness and biological relevance of the conditioned stimulus. *Archives of Sexual Behavior* 33, 43–53.

Jansen, A. (1998). A learning model of binge eating: cue reactivity and cue exposure. *Behaviour Research & Therapy* 36, 257-272.

Kafka, M.P. (2007). Paraphilia-Related Disorders: The evaluation of treatment of nonparaphilic hypersexuality. In: Leiblum R, editor. Principles and practice of sex therapy. 4 ed. New york: The Guilford Press; 2007, p. 442-77.
Kafka, M.P. (2010). Hypersexual disorder: a proposed diagnosis for DSM-V. *Archives of Sexual Behavior* 39, 377-400.
Kalisch R, Korenfeld E, Stephan KE, Weiskopf N, Seymour B, Dolan RJ. (2006). Context-dependent human extinction memory is mediated by a ventromedial prefrontal and hippocampal network. *Journal of Neuroscience* 26:9503-9511.
Kalisch, R., Holt, B., Petrovic, P., De Martino, B., Klöppel, S., Büchel, C., & Dolan, R. J. (2009). The NMDA Agonist D-Cycloserine Facilitates Fear Memory Consolidation in Humans. *Cerebral cortex* 19, 187-196.
Kindt, M., Soeter, M. & Vervliet, B. (2009) Beyond extinction: erasing human fear responses and preventing the return of fear. *Nature Neuroscience* 12, 256-258.
Klucken, T., Kruse O, Wehrum-Osinsky, S. Schweckendiek, J., Hennig, J., & Stark, R. (2015). Impact of COMT Val158Met polymorphism on appetitive conditioning and amygdala/prefrontal effective connectivity. *Human Brain Mapping*, 36, 1093-1101.
Laan, E., Everaerd, W., & Evers, A. (1995). Assessment of female sexual arousal: response specificity and construct validity. *Psychophysiology* 32, 476-485.
Martin-Soelch, C., Linthicum, J., and Ernst, M. (2007). Appetitive conditioning: neural bases and implications for psychopathology. *Neuroscience & Biobehavioral Reviews*, 31, 426–440. doi: 10.1016/j.neubiorev.2006.11.002
Mechias, M., Etkin, A., Kalisch, R. (2010). A meta-analysis of instructed fear studies: implications for conscious appraisal of threat. *Neuroimage* 49, 1760-1768.
Myers, K.M. & Carlezon, W.A. (2012). D-Cycloserine effects on extinction of conditioned responses to drug-related cues. *Biological Psychiatry* 71, 947–955.
Ori R, Amos T, Bergman H, Soares-Weiser K, Ipser JC, Stein DJ. (2015). Augmentation of cognitive and behavioural therapies (CBT) with d-cycloserine for anxiety and related disorders. *Cochrane Database of Systematic Reviews* 5; CD007803.
Pfaus, J.G., Kippin, T.E., & Centeno, S. (2001). Conditioning and sexual behavior: a review. *Hormones & Behavior* 40, 291–321.
Price, K.L., Baker, N.L., McRae-Clark, A.L., Saladin, M.E., Desantis, S.M., Santa Ana, E.J., & Brady, K.T. (2013). A randomized, placebo-controlled laboratory study of the effects of D-cycloserine on craving in cocaine-dependent individuals. *Psychopharmacology* 226, 739 –746.
Reichelt, A.C. & Lee, J.L.C. (2013) Memory reconsolidation in aversive and appetitive settings. *Frontiers in Behavioral Neuroscience*, doi:10.3389/fnbeh.2013.00118.
Rescorla, R. A., & Wagner, A. R. (1972). In A.H. Black & W.F. Prokasy (Eds.), Classical conditioning II: Current research and theory, 64-99. New York: Appleton-Centrury-Crofts.
Ressler KJ, Rothbaum BO, Tannenbaum L, Anderson P, Graap K, Zimand E, Hodges L, & Davis, M. (2004). Cognitive enhancers as adjuncts to psychotherapy: use of D-cycloserine in phobic individuals to facilitate extinction of fear. *Archives of General Psychiatry* 61, 1136 –1144.
Robinson, T. E. & Berridge, K. C. (1993). The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Research Reviews* 18, 247-291.

Rosen, R., Brown, C., Heiman, J., Leiblum, S., Meston, C., et al. (2000). The Female Sexual Function Index (FSFI): a multidimensional self-report instrument for the assessment of female sexual function. *Journal of Sex & Marital Therapy* 26, 191-208.
Rouaud, E. & Billard, J.M. (2003). D-cycloserine facilitates synaptic plasticity but impairs glutamatergic neurotransmission in rat hippocampal slices. *British Journal of Pharmacology* 140, 1051–1056.
Saez, A., Rigotti, M., Ostojic, S., Fusi, S., Salzman, C. D. (2015). Abstract context representations in primate amygdala and prefrontal cortex. *Neuron* 87, 869–881.
Santa Ana, E.J., Rounsaville, B.J., Frankforter, T.L., Nich, C., Babuscio, T., Poling, J., Gonsai, K., Hill, K.P., & Carroll, K. M. (2009). D-Cycloserine attenuates reactivity to smoking cues in nicotine dependent smokers: a pilot investigation. *Drug & Alcohol Dependence* 104, 220- 227.
Scavio, M.J., Clift, P.S., & Wills, J.C. (1992). Posttraining effects of amphetamine, chlorpromazine, ketamine, and scopolamine on the acquisition and extinction of the rabbit's conditioned nictitating membrane response. *Behavioral Neuroscience* 106, 900-908.
Schiller, D., Kanen, J. W., LeDoux, J. E., Monfils, M.H., Phelps, E. (2013). Extinction during reconsolidation of threat memory diminishes prefrontal cortex involvement. *PNAS* 110, no. 50, http://www.pnas.org/content/110/50/20040.
Schultz, W., Dayan, P., & Montague, P.R. (1997). A neural substrate of prediction and reward. *Science* 275, 1593–1599.
Schultz, W. (2006). Behavioral theories and the neurophysiology of reward. *Annual Review of Psychology* 57, 87–115.
Ter Kuile, M.M., Brauer, M., & Laan, E. (2006). The Female Sexual Function Index (FSFI) and the Female Sexual Distress Scale (FSDS): psychometric properties within a Dutch population. *Journal of Sex & Marital Therapy* 32, 289-304.
Ter Kuile MM, Both S, & van Lankveld J. (2009). Sexual dysfunctions in women. In: Sturmey P, Hersen M, editors. Handbook of Evidence-Based Practice in Clinical Psychology Volume II: Adult Disorders.
Todd, T.P., Vurbic, D., & Bouton, M.E. (2014). Mechanisms of renewal after the extinction of discriminated operant behavior. *Journal of Experimental Psychology- Animal Learning and Cognition* 40, 355-368.
Torregrossa, M.M., Gordon, J., & Taylor, J.R. (2013). Double Dissociation between the Anterior Cingulate Cortex and Nucleus Accumbens Core in Encoding the Context versus the Content of Pavlovian Cocaine Cue Extinction. *Journal of Neuroscience* 33, 8370–8377.
Torregrossa, M. M., Sanchez, H., & Taylor, J. R. (2010). D-Cycloserine reduces the context specificity of pavloian extinction of cocaine cues through actions in the nucleus accumbens. *Journal of Neuroscience* 30, 10526-10533.
Van Berckel, B.N., Lipsch, C., Gispen-de Wied, C., Wynne, H.J., Blankenstein, M.A., et al. (1998). The partial NMDA agonist D-cycloserine stimulates LH secretion in healthy volunteers. *Psychopharmacology (Berl)* 138,190-197.
Vurbic, D., Gold, B., & Bouton, M. E. (2011). Effects of D-cycloserine on the extinction of appetitive operant learning. *Behavioral Neuroscience*, 125, 551-559.
Wise, R.A. (2002). Brain reward circuitry: insights from unsensed incentives. *Neuron* 36, 229–240.

Woods AM, & Bouton, ME. (2006). D-cycloserine facilitates extinction but does not eliminate renewal of the conditioned emotional response. *Behavioral Neuroscience* 120, 1159–1162.
Zhu, M., Nix, D.E., Adam, R.D., Childs, J.M., & Peloquin, C.A. (2001). Pharmacokinetics of cycloserine under fasting conditions and with high-fat meal, orange juice, and antacids. *Pharmacotherapy* 21,891—897.