Neural Activations of the Acquisition of Conditioned Sexual Arousal: Effects of Contingency Awareness and Sex

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DOI: 10.1111/j.1743-6109.2009.01405.x

ABSTRACT

Introduction. Learning processes like classical conditioning are involved in mediating sexual behavior. Yet, the neural bases underlying these processes have not been investigated so far.

Aim. The aim of this study was to explore neural activations of classical conditioning of sexual arousal with respect to sex differences and contingency awareness.

Methods. In the acquisition phase, a geometric figure (CS+) was presented for 8 seconds and was followed by highly sexual arousing pictures (UCS), whereas another figure (CS–) predicted neutral pictures. Ratings and contingency awareness were assessed after the entire conditioning procedure. Forty subjects (20 females) were classified into one of four groups according to their sex and the development of contingency awareness (aware females, aware males, unaware females, and unaware males).

Main Outcome Measures. Blood oxygen level dependent (BOLD) responses measured by functional magnetic resonance imaging (fMRI), skin conductance responses (SCRs), and subjective ratings.

Results. fMRI analysis showed two effects (awareness and sex) when comparing CS+ with CS–: (i) aware compared to unaware subjects showed enhanced differentiation (e.g., ventral striatum, orbitofrontal cortex, occipital cortex); and (ii) men showed increased activity compared to women in the amygdala, thalamus, and brainstem. CS+ and CS– ratings differed in aware subjects only. However, no conditioned SCRs occurred in any group.

Conclusion. The increased activity in men is in line with theories postulating that men are generally more prone to conditioning of sexual arousal. Further, contingency awareness seems to be an important factor in appetitive learning processes, which facilitates conditioning processes. **Klucken T, Schweckendiek J, Merz CJ, Tabbert K, Walter B, Kagerer S, Vaitl D, and Stark R. Neural activations of the acquisition of conditioned sexual arousal: Effects of contingency awareness and sex. J Sex Med **;**:**–**.**

Key Words. Appetitive; Classical Conditioning; Female Sexual Response; Sexual Arousal; Ventral Striatum; Gender Differences

Introduction

Several theories postulate that learning experiences are essential for sexual behavior [1,2]. One way to investigate such learning processes is the use of classical conditioning paradigms. In a differential classical conditioning paradigm, a neutral stimulus (CS+) is paired with a salient unconditioned stimulus (UCS), whereas a second neutral stimulus (CS–) predicts its absence, or in case of picture–picture conditioning a neutral pictures (non-UCS). After just a few trials, the CS+ elicits conditioned responses (CRs), such as increased skin conductance responses (SCRs), increased brain activations [3,4], or in case of conditioning of sexual arousal even increased genital responses [5,6].

Classical conditioning of fear is already understood in considerable detail, yet research on appetitive conditioning is still very rare, even though appetitive conditioning is also evolutionary relevant [7]. In appetitive conditioning, a conditioned stimulus (CS) is paired with a pleasant UCS, e.g., an enjoyable odor [8] or monetary reward [9]. A specific form of appetitive conditioning is conditioning of sexual arousal. Typically,

visual stimuli like erotic pictures or short movie clips are used as UCS, but recently, Both et al. [5,6] employed a new and sophisticated approach by using direct genital stimulation (vibrotactile stimulation) as UCS. Irrespective of the UCS input modality, these studies reported robust CRs, e.g., increased genital responses [5,6,10].

To date, only few imaging studies have been undertaken to reveal the brain structures involved in appetitive conditioning. Reviewing the recent literature, Martin-Soelch et al. [7] identified several distinct brain structures that play an important role for appetitive conditioning: amygdala, orbitofrontal cortex (OFC), anterior cingulate cortex (ACC), brainstem, thalamus, occipital cortex, ventral tegmental area (VTA), and ventral striatum.

One of the most studied brain structures in classical conditioning is the amygdala, which for example mediates and elicits CRs such as heart rate and hormonal responses [11,12]. OFC and ACC activations reflect the conscious subjective evaluation of stimuli, for instance, the anticipation of future emotional events [13,14]. In their review, Day and Carelli [15] highlight the role of the ventral striatum, or more specifically the nucleus accumbens and the VTA as key regions for appetitive conditioning. A central role of the ventral striatum in appetitive conditioning is also suggested by studies showing its involvement in the processing and the anticipation of positive events [15]—especially sexual arousal [16,17]—as well as by research highlighting its role in craving and addiction learning processes [18]. Finally, the brainstem, the thalamus, the insula, and the occipital cortex are involved in the processing of emotional stimuli and in behavioral (motor) responses [7,17].

In the field of appetitive conditioning, two important factors, which might influence CRs, are discussed: sex and contingency awareness. Regarding the influence of sex, mainly investigated by animal studies, many authors propose that males compared to females are more susceptible to classical conditioning of sexual arousal, resulting in increased and/or facilitated CR acquisition [1,2,19]. Most human studies have focused on only one sex. To our knowledge, differences between women and men have only been directly investigated in one study [10]. Furthermore, investigating the neural correlates of human female sexual responses is only beginning to be studied. First results suggest that, in general, the same brain mechanisms are involved in women and men (for a detailed insight in the neural bases of sexual arousal, see [20,21]). Regarding contingency awareness, a heated debate about its influence in classical conditioning is still going on—in aversive as well as in appetitive conditioning (cf. [22–24]). Contingency awareness can be defined as the explicit knowledge about the CS–UCS relationships and the conscious differentiation between CS+ (as the predictor for the salient UCS) and CS–. Some studies found contingency awareness to be necessary for the development of CRs [13,25–27], whereas other authors observed CRs without contingency awareness [28–30]. These inconsistencies might partly be caused by methodological differences in the conditioning paradigms. In some studies, contingency awareness is prevented by subliminal stimulus presentations, in which the CS are presented very briefly (approximately 30 ms) and masked by a second stimulus (backward masking) [6,29,30]. In other studies, the CS are presented supraliminally (i.e., several seconds), and contingency awareness is not explicitly manipulated [13,31]. Thus, in these studies some of the participants develop contingency awareness, whereas others do not.

The aim of the present study was to investigate the neural activations underlying classical conditioning of sexual arousal. Furthermore, we also examined sex differences and the response patterns of contingency aware and unaware subjects. To examine these questions, the subjects were classified into four groups according to their sex and the development of contingency awareness during the experiment (i.e., aware females, aware males, unaware females, and unaware males). We analyzed three different response systems: subjective ratings, electrodermal activity (SCRs), and neural activity.

Regarding the subjective ratings of the CS, we hypothesized in line with the literature (e.g., [13,25,27]) that aware subjects would rate the CS+ as more pleasant, as more generally arousing, and as more sexually arousing compared to the CS–, whereas unaware participants would not show such differences. Regarding SCRs, we assumed that only aware subjects would exhibit conditioned SCRs, whereas all subjects would show higher SCRs to the UCS compared to the non-UCS. Regarding brain activity, we hypothesized that contingency awareness and sex would influence the learning process at least regarding the following two aspects: (i) contingency aware compared to unaware subjects would show increased hemodynamic responses to the CS+ than to the CS– in structures known to be involved in appetitive conditioning; and (ii) men would show increased responses in the contrast CS + > CS– compared to women.

Method

Participants

Forty-four heterosexual subjects (22 women) with a mean age of 23.05 (SD = 3.27) years participated in the study. None of them had a history of psychiatric or neurological disorders. All subjects were right handed and had normal or corrected-to-normal vision. They received 16 Euros for their participation. The majority of participants were students who responded to announcements posted at the campus of the University of Giessen. The participants were informed about the procedure in general (but not about the conditioning paradigm, until the experiment was finished). They signed an informed consent, which stated that they could terminate the experiment at any time. The study was conducted in accordance with the Declaration of Helsinki, and was approved by the ethics committee of the German Psychological Society.

CS

Two visual geometric shapes (a rhomb and a square) served as CS+ and CS–. All stimuli were gray, had identical luminance, and were presented in an 800 × 600 pixel resolution. The stimuli were projected onto a screen at the end of the scanner (visual field = 18°) using an LCD projector. Pictures were viewed through a mirror mounted on the head coil.

UCS

For the acquisition phase, a set of 21 sexually arousing pictures were presented as UCS. The erotic pictures depicted scenes with couples (always one man and one woman) practising vaginal intercourse in different positions, as well as oral and manual stimulation. These pictures were already used in previous studies and rated as highly sexually arousing. The neutral pictures (non-UCS) depicted men and women in neutral scenes. All (erotic and nonerotic) pictures were presented in color and had identical pixel resolution. Five of the erotic stimuli and two of the neutral stimuli were taken from the International Affective Picture System [32]. All other stimuli were collected by the authors from the Internet.

Assessment of Contingency Awareness

Immediately after the entire conditioning procedure, contingency awareness was measured by a short recognition questionnaire. The question asked was: "Which geometric figure was presented before the sexual pictures?" The subjects had to choose one of the following statements: "There was. . . ." (i) "a rhomb;" (ii) "a square;" or (iii) "I do not know." Furthermore, an interview was conducted immediately after the experiment in which subjects had to explicitly verbalize the association between the CS+ and UCS, as well as between the CS– and the non-UCS. For classification as contingency aware, subjects had to state the correct answer in the recognition questionnaire and in the interview. For classification as unaware, the subjects had to state "I do not know." Subjects were excluded from further analyses if they associated the CS– with the UCS. Overall, we were interested in comparing subjects, who learned the correct associations between CS and the following pictures (UCS) and subjects, who had no idea that such associations exist. In case of wrong associations, it is not clear what kind of expectancies were developed by the subjects. They might have developed a cognitive construct about the contingencies and therefore differ from both the unaware and the aware groups. Answers in the questionnaire and the interview never diverged.

Rating of the CS and UCS

After the experiment, the participants rated subjective valence and arousal with the self-assessment manikin [33], as well as sexual arousal and disgust for each stimulus (CS+, CS–, UCS, non-UCS) on a 9-point Likert scale. All scales ranged from "1" (e.g., indicating: not disgusting at all, unpleasant, etc.) to "9" (e.g., indicating: very disgusting, very pleasant, etc.). Statistical analyses were performed via analyses of variance (ANOVAs) in a 2 (stimulus type: CS+ vs. CS–) × 2 (awareness: unaware vs. aware) × 2 (sex: male vs. female) factorial design in the general linear model (GLM) as implemented in SPSS 17 for Windows (SPSS Inc., Chicago, IL, USA).

Conditioning Procedure

The conditioning procedure contained an acquisition phase and an extinction phase. In the acquisition phase, 42 trials were presented (each CS 21 times). The CS duration was 8 seconds. The UCS appeared immediately after the CS (100% reinforcement) for 4 seconds. Contrary to fear conditioning (e.g., with electrical stimulation), in which the non-UCS is shock omission, we used neutral pictures as non-UCS. This procedure is the most common approach in the field of picture–picture conditioning (e.g., [27,34]). We also wanted to investigate brain structures underlying the processing of visual sexual stimuli. Therefore, we

required an adequate comparison condition to control for the influences of visual processing per se. The intertrial intervals ranged from 5.5 to 8 seconds. The rhomb and the square were counterbalanced as CS+ across all participants.

The extinction phase contained 22 trials (each CS type 11 times) with the same stimulus and intertrial interval duration as in the acquisition phase, but without reinforcement (a black screen was presented instead of the UCS/non-UCS). The present study focused on the acquisition of appetitive responses. Thus, only the results of the acquisition phase are presented. After the entire conditioning procedure, contingency awareness and subjective ratings were assessed. The decision to measure contingency awareness after the extinction phase was made in order to avoid influencing the extinction phase with contingency awareness assessment and subjective ratings between the acquisition and the extinction phase. Several studies have shown that subjective ratings and contingency awareness are unaffected by a short extinction phase [35,36]. For each subject, a pseudo-randomized stimulus order was used with the restrictions that: (i) no more than two presentations of the same CS succeeded; and that (ii) the CS were equally distributed within half of the acquisition; respectively, the extinction phase. Throughout the experiment, eye movements were recorded by an MRI-compatible video camera to control if subjects watched the stimuli.

Skin Conductance Measuring

SCRs were sampled simultaneously with MR scans using Ag/AgCl electrodes filled with isotonic (0.05 M NaCl) electrolyte medium, placed hypothenar at the nondominant (left) hand. SCRs were defined in three analysis windows [37]: the maximum response (amplitude) within the time window 1–4 seconds after the CS (CS+ or CS–) onset was counted as the first interval response (FIR), within the time window 4.5–8 seconds as the second interval response (SIR), and within the time window 8.5–12 seconds as the unconditioned response (third interval response). The response amplitudes were computed as the differences between the minimum before and the maximum after a point of reflection. Responses were only registered when the response amplitude was greater than 0.01 μs. A logarithmic transformation was conducted to ensure comparability between the subjects. The mean of the responses was explored separately in two blocks with each block containing half of the CS+ and CS– presentations (i.e., 10 trials in the acquisition and five trials in the extinction phase). Statistical analyses were performed via ANOVA in a 2 (stimulus type) × 2 (block) × 2 (awareness) × 2 (sex) factorial design implemented in SPSS 17.

Magnetic Resonance Imaging

Functional and anatomical images were acquired with a 1.5 tesla whole-body tomograph (Siemens Synphony [Erlangen, Germany], with a quantum gradient system) with a standard head coil. Structural images acquisition consisted of 160 T1-weighted sagittal images (MPRage, 1 mm slice thickness). For functional images, a total of 502 images were registered using a T2*-weighted gradient echo-planar imaging (EPI) sequence with 25 slices covering the whole brain (slice thickness = 5 mm; 1 mm gap; descending slice procedure; TR = 2.5 s; TE = 55 ms; flip angle = 90°; field of view 192 × 192 mm; matrix size = 64 × 64). The orientation of the axial slices was parallel to the OFC bone transition in order to minimize susceptibility artifacts in prefrontal areas. Data were analyzed using Statistical Parametric Mapping (SPM2, Wellcome Department of Cognitive Neurology, London UK; 2002) implemented in MATLAB 6.5 (Mathworks Inc., Sherborn, MA, USA). Prior to all analyses, data were preprocessed; this included realignment (b-spline interpolation), slice time correction, normalization to the standard brain of the Montreal Neurological Institute (MNI-EPI-template), and smoothing with an isotropic three-dimensional Gaussian filter with a full width at half maximum of 9 mm. The experimental conditions were CS+, CS–, UCS, and non-UCS. Regressors were convolved with a hemodynamic response function in the GLM. The six movement parameters of the rigid body transformation applied by the realignment procedure were introduced as covariates in the model. The voxel-based time series was filtered with a high pass filter (time constant = 128 seconds).

The following contrasts were analyzed by ANOVA implemented in SPM2: CS+ > CS– and CS– > CS+, as well as UCS > non-UCS and non-UCS > UCS. Difference scores of these contrasts (e.g., CS+ > CS–) were calculated for each subject and introduced as dependent variable in the group analyses (second-level analyses). Sex and contingency awareness were introduced as group factors. To test for the main effect of CS type (or UCS type), we calculated the mean for these contrasts (e.g., CS+ > CS–) for all subjects. To test for the effects of awareness and sex on conditioning

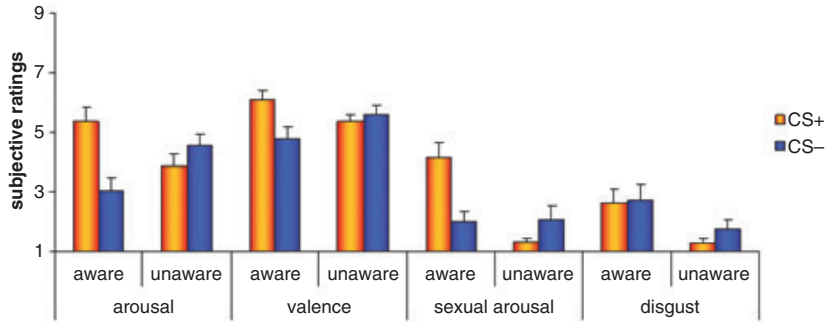

Figure 1 Mean (and SEM) of the subjective ratings for the CS+ and CS– in the aware and unaware groups.

related brain activation, we calculated the interactions between sex and CS type (or UCS type), as well as awareness and CS type (or UCS type) by comparing the difference scores between the groups. For example, a potential interaction effect of contingency awareness with CS type was analyzed by comparing aware males and aware females with unaware males and females. Finally, a threefold interaction awareness × sex × CS type (or UCS type) was analyzed by comparing the four groups. Appropriate post hoc tests were calculated for significant main effects and interactions.

Regions of interest (ROIs) analyses were performed using the small volume correction in SPM2 ($P < 0.05$ for significant results and $P < 0.10$ for trends, family-wise error [FWE]-corrected). A minimum cluster size of 5 voxel was required. The ROIs were the amygdala, ACC, brainstem, insula, medial OFC, occipital cortex, thalamus, ventral striatum, and VTA. All masks except the VTA mask were probability masks taken from the current "Harvard–Oxford cortical and subcortical structural atlases" provided by the Harvard Center for Morphometric Analysis (http://www.cma.mgh.harvard.edu/) and from the Human Brain Project Repository database (THOR Center for Neuroinformatics; http://hendrix.ei.dtu.dk) based on the BrainMap database [38,39] with the probability threshold at 0.5. Because of the lack of a VTA mask in the mentioned atlases, this ROI was created with MARINA [40]. The creation of masks in MARINA is aided by the anatomical parcellation of the brain published by Tzourio-Mazoyer et al. [41].

Results

Four participants were excluded from the analyses: two subjects stated wrong contingencies between CS and UCS in the recognition questionnaire, as well as in the interview (i.e., they stated that the UCS were presented after the CS–). Two participants closed their eyes during the acquisition phase for more than five trials. Hence, data of 40 participants were included in the final analyses and in accordance to their awareness rating, the subjects were classified as unaware or as aware: nine subjects were categorized as aware females, 11 as unaware females, 11 as aware males, and nine as unaware males.

Effects of the CS (CS+ > CS–)

Subjective Ratings

We found a significant main effect of CS type in the arousal ratings ($F_{(1,36)} = 4.28$; $P < 0.05$) with higher responses to the CS+ compared to the CS–. Trends for main effects of CS type were observed for valence ($P = 0.065$) and sexual arousal ($P = 0.079$) ratings. We also found interaction effects between CS type and awareness in valence ($F_{(1,36)} = 6.01$; $P < 0.05$), arousal ($F_{(1,36)} = 16.27$; $P < 0.001$), and sexual arousal ($F_{(1,36)} = 16.14$; $P < 0.001$) ratings, but not in disgust ratings. Post hoc *t*-tests showed significant differences in arousal, valence, and sexual arousal ratings between CS+ and CS– in the aware group, but not in the unaware group (see Figure 1). No further main or interaction effects occurred.

SCRs

The ANOVA only showed a main effect of block (first vs. second half) in the FIR ($F_{(1,35)} = 4.30$; $P < 0.05$) and no effects in the SIR. FIR amplitude decreased significantly over time, but no other main or interaction effects occurred.

Hemodynamic Responses

The ANOVA revealed effects of CS type, awareness, and sex in the ventral striatum, medial OFC, occipital cortex, VTA, brainstem, and thalamus in the contrast CS+ > CS– (all *F* values > 9). In order to determine the direction of these effects, post hoc *t*-tests were computed for these regions to

Table 1 Results from post hoc tests for determining the direction of the effect of awareness and sex in the contrast CS+ > CS– and one sample *t*-tests for the aware and unaware group, respectively, male and female subjects

Effects	Group	Brain structure	*x*	*y*	*z*	T_{max}	P_{corr}
Awareness × CS type (CS+ > CS–)	Aware > unaware	L Medial OFC	–21	21	–18	4.64	0.008
		R Occipital cortex	45	–72	–15	3.58	0.046
		L Ventral striatum	–15	15	–15	3.41	0.022
		R Ventral striatum	12	12	–12	2.96	0.057
		VTA	0	–18	–12	2.66	0.044
	Unaware > aware	No significant activations					
One-sample *t*-test	Aware group	L ACC	–3	0	36	6.59	0.007
		R ACC	6	0	36	4.87	0.035
		L Amygdala	–15	–3	–18	3.53	0.035
		L Insula	–39	9	0	5.75	0.001
		R Insula	39	9	–9	4.36	0.015
		L Occipital cortex	–48	–66	–6	4.43	0.030
		L Ventral striatum	–12	3	–3	3.56	0.016
		R Ventral striatum	15	3	–12	3.25	0.071
	Unaware group	No significant activations					
Sex × CS type (CS+ > CS–)	Male > female	R Amygdala	33	0	–18	3.47	0.017
		L Brainstem	–3	–42	–39	4.46	0.012
		R Thalamus	9	–18	18	3.53	0.034
		L Occipital cortex	–48	–63	–6	3.29	0.082
		R Occipital cortex	54	–63	0	3.77	0.031
	Female > male	No significant activations					
One-sample *t*-test	Male subjects	R Brainstem	–3	–42	–39	3.95	0.083
		R Thalamus	9	–18	18	3.87	0.033
		L Occipital cortex	–51	–63	–3	4.30	0.023
		R Occipital cortex	54	–60	0	3.60	0.078
		L Ventral striatum	–9	6	–3	3.18	0.052
	Female subjects	No significant activations					

The threshold was $P < 0.05$; for trends $P < 0.10$ (ROI analyses; FWE-corrected; small volume correction according to SPM2). All coordinates are given in MNI space. L = left; R = right hemisphere.

analyze if aware or unaware subjects (or in case of sex males or females) showed greater differentiation in hemodynamic responses in the contrast CS+ > CS–. For the whole group, we found a main effect of CS type in the occipital cortex ($t = 3.84$; $P < 0.05$; coordinates: $x = -48$, $y = -63$, $z = -3$) and trends in the ventral striatum ($t = 2.81$; $P = 0.075$; coordinates: $x = -12$, $y = 6$, $z = -6$), and in the insula ($t = 3.10$; $P = 0.063$; coordinates: $x = -33$, $y = 21$, $z = -3$) with greater activations toward the CS+ compared to the CS–. We also found an interaction effect of awareness × CS type. Contingency aware subjects compared to unaware subjects showed significantly greater differentiation in hemodynamic responses in the contrast CS+ > CS– in the medial OFC, VTA, occipital cortex, left ventral striatum, and a trend in the right ventral striatum (Table 1; Figure 2). Further, interaction effects of sex × CS type were observed in the amygdala, brainstem, thalamus, left occipital cortex, and a trend in the right occipital cortex with higher responses in men compared to women.

These main effects and interactions were further elucidated via *t*-tests within the respective groups (i.e., CS+ > CS– in the aware, unaware, male, female groups). We found enhanced differentiation (CS+ > CS–) in contingency aware subjects in the ACC, amygdala, insula, occipital cortex, and ventral striatum. No significant activa-

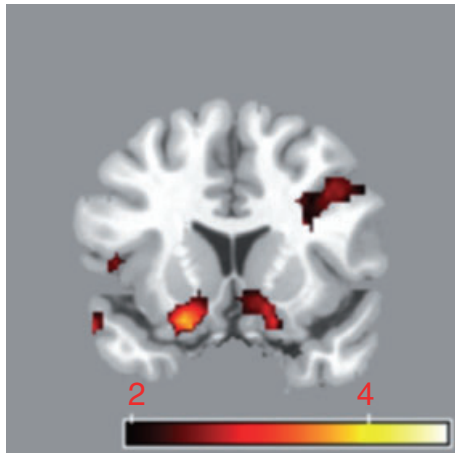

Figure 2 Neural activation (in *T*-values) of the group comparison aware > unaware for the left and the right ventral striatum in the contrast CS+ > CS–. For illustration reasons, the data are shown with a threshold at *T* value > 2.50. Statistical parametric maps are overlaid on a T1 template (depicted from the SPM2 package).

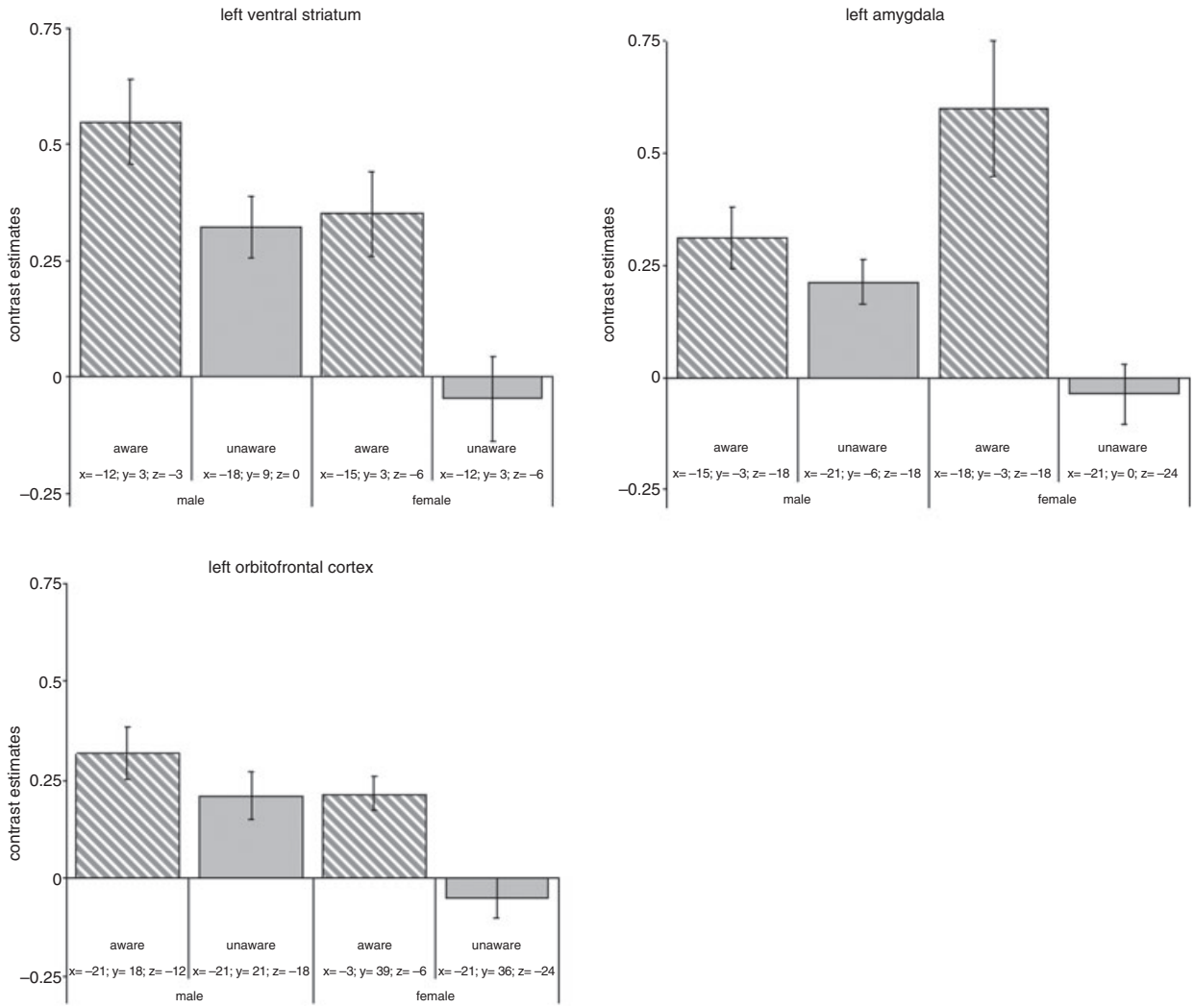

Figure 3 Mean (SEM) of the contrast estimates in the ventral striatum, amygdala, and orbitofrontal cortex (CS+ > CS–) for the peak voxels from each group separately.

tions occurred in unaware subjects. Further significant differentiations in the male group for the contrast CS+ > CS– were found in the thalamus, left occipital cortex, and trends in the brainstem, left ventral striatum, and right occipital cortex. No significant activations or trends occurred in the female group.

We further analyzed the responses toward CS+ and CS– separately in the groups with respect to the significant CS+ > CS– contrasts. These analyses revealed that these significances resulted mainly from increased CS+ responses and not from decreased CS– responses. Analyzing each of the four groups separately (i.e., aware males, unaware males, aware females, unaware females), we found increased activity in the ventral striatum, occipital cortex, insula, and ACC in the aware-male group (for details of contrast estimates, see Figure 3). In the aware-female group, we found a similar response pattern (significant activation in the occipital cortex and trends in the insula, ACC), and additionally a trend in the amygdala. For the other two groups, we did not find increased activations (see Table 2).

Following a worthwhile reviewer's comment, we investigated whether the greater differentiation between the CS+ and CS– in the aware compared to the unaware subjects was caused by the higher sexual arousal ratings of the UCS in the aware compared to the unaware subjects. An ANOVA with these ratings as covariate revealed only marginally changed activation patterns (i.e., all significant values remained $P < 0.05$, and no additional structures showed significant P values).

Table 2 Neural activations for the contrast CS+ > CS– for each group separately

Group	Brain structure	*x*	*y*	*z*	T_{max}	P_{corr}
Aware males	L ACC	–6	–3	39	6.53	0.041
	L Insula	–42	3	0	5.11	0.028
	R Insula	39	0	0	5.18	0.026
	L Occipital cortex	57	–60	3	4.03	0.017
	L Ventral striatum	–12	3	–3	3.51	0.018
Aware females	L Amygdala	–18	–3	–18	2.42	0.095
	L Occipital cortex	–48	–63	–3	4.61	0.004
	R Ventral striatum	18	6	–9	4.61	0.081
Unaware males	No significant activations					
Unaware females	No significant activations					

The threshold was $P < 0.05$; for trends $P < 0.10$ (ROI analyses; FWE-corrected; small volume correction according to SPM2). All coordinates are given in MNI space. L = left; R = right hemisphere.

Effects of the UCS (UCS > Non-UCS)

Subjective Ratings

The ANOVA revealed significant main effects for the UCS type for valence ($F_{(1,36)} = 6.37$; $P < 0.05$), arousal ($F_{(1,36)} = 103.52$; $P < 0.001$), and sexual arousal ratings ($F_{(1,36)} = 82.90$; $P < 0.001$), showing higher ratings for the UCS than for the non-UCS (Figure 4). A main effect of sex was found in valence ($F_{(1,36)} = 6.05$; $P < 0.05$) and sexual arousal ($F_{(1,36)} = 9.62$; $P < 0.01$) showing higher responses of men compared to women. A significant interaction effect CS type × awareness was found, revealing higher sexual arousal ratings to the UCS in aware subjects compared to unaware subjects ($F_{(1,36)} = 7.92$; $P < 0.01$). No significant effects were found for disgust ratings.

SCRs

Two significant main effects for the UCS type ($F_{(1,35)} = 4.38$; $P < 0.05$) and block ($F_{(1,35)} = 5.79$; $P < 0.05$) occurred. SCR responses were greater toward the UCS compared to the non-UCS, and were higher in the first block compared to the second block of acquisition irrespective of sex and awareness.

Hemodynamic Responses

Again, the ANOVA revealed strong effects of awareness and sex in the contrast UCS > non-UCS for the hypothesized regions, which were again tested with post hoc tests to specify the direction of these effects (see Table 3). We found a significant main effect of UCS type for all mentioned ROIs. The ANOVA revealed a significant interaction effect awareness × UCS type in the VTA, and a trend in the ventral striatum with higher responses to the UCS compared to the non-UCS. Further, a significant interaction effect sex × UCS type was found in several structures (Table 3). In all mentioned regions, men showed greater differences in the contrast UCS > non-UCS as compared to women. These interactions were also elucidated via *t*-tests within the respective groups (i.e., UCS > non-UCS in the aware, unaware, male, female groups). One-sample *t*-tests revealed that men and women showed significantly enhanced activity toward the UCS compared to the non-UCS in most ROIs. For the aware and the unaware groups, the UCS resulted in more activation compared to the non-UCS in all ROIs.

Discussion

The present study investigated the neural activations of conditioning of sexual arousal, which has so far been neglected. The results provided two significant interaction effects: first, contingency

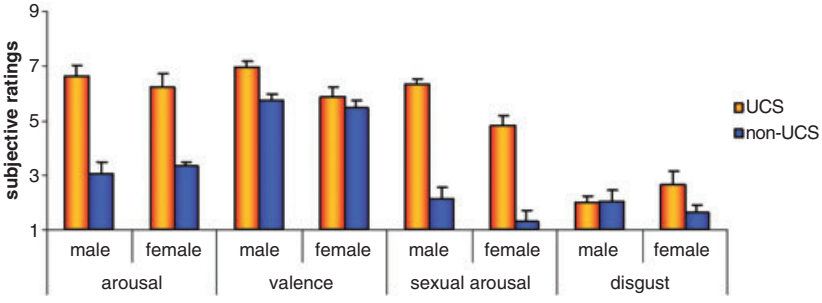

Figure 4 Mean (and SEM) of the subjective ratings for the unconditioned stimulus (UCS) and non-UCS in the aware and unaware groups.

Table 3 Results from post hoc tests for determining the direction of the effect in the contrast UCS > non-UCS of awareness and sex, and one sample *t*-tests (gray shaded) for the aware and unaware group, respectively, male and female subjects

Effects	Group	Brain structure	*x*	*y*	*z*	T_{max}	P_{corr}
Awareness × UCS type (UCS > non-UCS)	Aware > unaware	L Ventral striatum	–12	18	–9	3.04	0.056
		VTA	0	–18	–9	2.56	0.044
	Unaware > aware	No significant activations					
Sex × UCS type (UCS > non-UCS)	Male > female	L Brainstem	–9	–45	–57	4.12	0.033
		L Insula	–36	-3	12	4.12	0.007
		L Medial OFC	–24	24	–18	5.31	0.003
		R Medial OFC	9	54	–12	4.49	0.016
		R Occipital cortex	33	–57	3	3.71	0.042
		L Thalamus	–15	–15	9	4.22	0.008
		R Thalamus	3	–15	12	3.48	0.046
		L Ventral striatum	–15	6	–9	3.78	0.011
	Female > male	No significant activations					
One-sample *t*-test	Male subjects	L ACC	–3	3	39	6.44	0.001
		R ACC	3	18	24	6.50	0.001
		L Amygdala	–18	–9	–12	3.19	0.041
		R Amygdala	18	–12	–15	4.99	0.002
		L Brainstem	–9	–30	–9	4.50	0.029
		L Insula	–39	9	–12	5.07	0.003
		R Insula	39	12	–9	4.05	0.022
		L Medial OFC	–24	24	–18	5.85	<0.001
		R Medial OFC	21	24	–18	4.91	0.021
		L Thalamus	–3	–15	12	4.86	0.008
		R Thalamus	3	–15	12	4.85	0.009
		L Occipital cortex	–51	–69	–3	12.5	<0.001
		R Occipital cortex	51	–69	–12	11.2	<0.001
		L Ventral striatum	–12	6	–6	5.53	0.001
		R Ventral striatum	12	3	–3	5.56	0.001
	Female subjects	L ACC	–3	36	0	4.87	0.017
		L Amygdala	–27	0	–24	2.85	0.090
		R Amygdala	24	–12	–21	2.85	0.090
		L Insula	–36	12	–12	4.24	0.017
		R Insula	39	0	–12	6.25	<0.001
		L Medial OFC	–27	15	–18	4.87	0.006
		R Medial OFC	24	6	–18	5.29	0.050
		L Occipital	–48	–69	–6	13.1	<0.001
		R Occipital	48	–63	–9	12.1	<0.001
		L Ventral striatum	–9	6	–9	4.34	0.003
		R Ventral striatum	12	3	–3	3.31	0.032

The threshold was $P < 0.05$; for trends $P < 0.10$ (ROI analyses; FWE-corrected; small volume correction according to SPM2). All coordinates are given in MNI space. L = left; R = right hemisphere.

aware subjects as compared to unaware subjects showed greater hemodynamic responses in the ventral striatum, medial OFC, occipital cortex, and VTA in the contrast CS+ > CS–. Second, in the same contrast, men compared to women showed higher responses in the amygdala, thalamus, and occipital cortex. Before focusing on the functional magnetic resonance imaging results to the CS, we would like to briefly discuss the UCS findings, as well as conditioning related subjective ratings and SCRs.

The results for the contrast UCS > non-UCS replicated previous findings, revealing increased activity of reward-related structures (e.g., the ventral striatum, OFC, VTA) and structures more generally involved in the processing of emotional stimuli (e.g., the amygdala, insula, and occipital cortex) [7,17]. Furthermore, SCRs and subjective ratings including sexual arousal ratings reliably differentiated between UCS and non-UCS. This shows that the pictures used were indeed able to elicit positive emotions.

Turning to the subjective ratings, CS+ and CS– ratings differed in aware subjects only. These results are in accordance with a growing number of studies showing that contingency awareness is essential for differential ratings of the CS+ and CS– [6,13,25]. However, it should be noted that the influence of contingency awareness on subjective ratings is still being debated [22], because of contrary findings in contingency unaware subjects. These conflicting findings might be explained by differences in the experimental procedures and individual differences (e.g., number of different

CS, assessment of contingency awareness, individual goals) (e.g., [6,13,26,42]).

Regarding SCRs, we did not find CRs. Despite the fact that conditioned SCRs are a common finding in fear conditioning [4,13,29,43–45], studies focusing on classical conditioning of sexual arousal repeatedly failed to find SCR differentiation. For instance, Hoffmann et al. [10] did not find increased SCRs but conditioned genital responses. In the study by Both et al. [6], differences in SCRs only occurred in the first acquisition trial and disappeared after this trial. Again, genital responses showed more reliable results for conditioning [6]. In sum, one could assume that the anticipation of an aversive UCS (e.g., electric shocks) might lead to a higher sympathetic activation (i.e., resulting in higher SCRs) than the anticipation of an erotic stimulus. We assume that SCRs might not be a sensitive indicator for conditioning of sexual arousal compared to other physiological parameters like genital responses—at least with the rather short ITI used in the present study. For example, Lachnit et al. [46] showed that short ITIs might hamper good SCR discrimination, while longer ITIs would facilitate CS+/CS– differentiation.

Turning to the conditioning-related neural activity, the most important finding is the increased striatal activity in the contingency aware subjects when comparing CS+ and CS–. The striatum is currently one of the most discussed structures in the field of appetitive conditioning because of its prominent role in the processing of positive emotions, like reward anticipation, valence decoding, and maintaining motivation [7,15]. Further, several studies showed that the ventral striatum is critically involved in the formation of learned associations between CS and UCS. For instance, Setlow et al. [47] reported neural activity in the ventral striatum, while rats learned that an olfactory CS predicted (rewarding) sucrose but no activity occurred if the olfactory stimulus was presented without reinforcement. Therefore, it is not surprising that the striatum is a key region for addiction-related learning processes [18]. In detail, it is assumed that dopaminergic projections from the VTA might be responsible for the experience and the anticipation of positive emotions [15,18], which is in line with our findings of enhanced VTA activation. Besides this striatal involvement in positive emotions, recent studies suggest a more general role for the striatum in the development of contingency awareness, irrespective of UCS valence [48,49]. Schiller et al. [49] found increased striatal activity in aware subjects whenever contingencies changed. This result is in line with a recent study reporting striatal activity in fear conditioning only in subjects who learned the contingencies during the experiment, but not in subjects who were unaware or were informed about the contingencies in advance [48]. Analyzing recent animal studies, Day and Carelli [15] concluded that striatal activity increases during the forming of associations, but "if rewards are fully predicted by a CS, they no longer evoke activation among dopamine neurons" (p. 153). The strong effect of awareness on striatal activity observed in the present study supports the central role of the striatum in the formation of stimulus–reward association. Contrary to the studies mentioned, Pessiglione et al. [50] found increased striatal activity in unaware subjects using the backward masking paradigm. They hypothesized that the "human brain can learn rewarding values of the CS without consciousness." As mentioned in the Introduction, the effects of awareness on CRs differ with respect to subliminal and supra-threshold CS presentation (cf. [29,45]).

In addition to this main finding, we found greater responses toward the CS+ compared to the CS– in contingency aware than unaware subjects in the medial OFC and the occipital cortex. The OFC has often been linked to the evaluation and the anticipation of the affective value of stimuli (for review, see: [51]). The medial OFC might be involved in the evaluation of positive emotions and anticipation of reward. Further support for this involvement of the medial OFC is provided by Gottfried et al. [8]. They conducted simultaneous appetitive and aversive conditioning, and found that the medial OFC responded more to the CS+, which predicted a pleasant UCS (odor), whereas activation in the lateral OFC was greater to the CS, which predicted an aversive UCS. Further, we also found increased occipital activity, which is a well-replicated finding in classical conditioning and is thought to reflect increased salience of the CS+ [4,13].

An arising question concerns the causal relation between contingency awareness and differences in unconditioned responses. We found higher sexual arousal ratings of the UCS, as well as greater differentiation in hemodynamic responses in the contrast UCS > non-UCS in the ventral striatum and the VTA in the aware compared to the unaware group. One might speculate that these higher unconditioned responses signal an enhanced salience of the UCS facilitating the

formation of contingency awareness. Alternatively, several authors assume a reverse causal relationship, namely that contingency awareness modulates the unconditioned responses. For instance, Domjan [1] argued that one of the most important functions of conditioning (and contingency awareness) is to react fast and adequately to the UCS. In line with this view, several studies found different UCS responses with respect to awareness (e.g., [1,52]).

Analyses of the interaction effects sex × CS type revealed a greater differentiation in the contrast CS+ > CS– in the amygdala, brainstem, thalamus, and occipital cortex in men compared to women. This is in line with current findings suggesting that men are more receptive to conditioning of sexual arousal than women even across different species (e.g., [2,19]). Sex differences in conditioning of sexual arousal (CS+ > CS–), as well as in processing of sexual stimuli (UCS > non-UCS), might be driven by biological factors [53–56] or by sociocultural factors. Regarding the biological factors, a large amount of evidence especially from animal research showed sex-related structural and functional differences in the brain, which are relevant for the processing of sexual stimuli (e.g., [56]). Our finding of higher right amygdala activation in men compared to women fits well with the finding by Killgore and Yurgelun-Todd [57], who also reported higher right amygdala activation in men in response to appetitive stimuli. In addition, Canli and Gabrieli [58] in their review pointed out that the greater amygdala activity in men cannot solely be explained by higher subjective arousal ratings of erotic stimuli. In keeping with the hypothesis of a central role of the amygdala in appetitive conditioning, this might account for the assumption that men are more receptive to conditioning of sexual arousal than women. Further, sex differences can also be found in various neurotransmitter systems, for instance serotonin, acetylcholine, dopamine, and also in opioids [53]. Current studies associated testosterone [59] and cortisol [60] with sexual arousal and its disorders, and also with the acquisition of CRs during classical conditioning [61,62]. All in all, it can be assumed that neurotransmitters and hormones affect reward learning by altering neural activity (e.g., in the amygdala and ventral striatum [53,63,64]).

Regarding sociocultural influences, there might be factors that facilitate the processing of sexual stimuli in men and inhibit it in women (i.e., like less acceptance of pornographic materials, more shame, etc. [65]). Current results showed that CRs can be inhibited by emotion regulation strategies like avoiding or reappraisal even in neural activations [66].

Aside from biological and sociocultural factors, the used paradigm might be more suitable in appetitive conditioning with sexual stimuli in men than in women. For example, women might be slower in returning to the baseline and thus require longer ITIs. Further, pictures as UCS might be less effective in women, whereas other UCS modalities like genital stimulation lead to strong CRs in women [5,6,10].

Interestingly, regarding the influence of sex and awareness, we found a dissociation of the different response levels (i.e., no sex differences in subjective ratings and electrodermal activity, but in hemodynamic responses). Other studies investigating the influence of awareness and sex also report dissociations between different response levels (e.g., [13,67,68]). For instance, Klucken et al. [13] did not find CRs in subjective ratings in unaware but in aware subjects. In contrast, regarding hemodynamic responses to the CS, unaware as well as aware subjects showed increased responses toward the CS+. A dissociation of the response levels has also been reported when contrasting men and women. For example, in an observational aversive conditioning procedure, Kelly and Forsyth [67] found no differences in the physiological responses to the CS but in subjective ratings. In a current review, Dalla and Shors [68] also highlight the impact of sex on different response levels in several conditioning procedures. In sum, the results suggest that sex and awareness might influence the various response levels differently.

Some limitations of the present study should be noted. First, contingency awareness was assessed after the extinction phase. This approach was chosen because ratings and questionnaires between the two phases might influence the following extinction phase. Although some studies have shown contingency awareness to still occur after short extinction phases [36,69], misclassification cannot be ruled out. However, misclassification would probably have occurred in one direction only (aware subjects forgetting the contingencies and hence, being classified as unaware) resulting in a possible misinterpretation of effects in the unaware group. Second, one could argue that the employed supra-threshold CS presentation might not be optimal to produce CRs in unaware subjects. CRs in unaware subjects have

more often been observed with subliminal CS presentation (e.g., [6,29]). Despite this, we chose a supra-threshold CS presentation to enable a thorough perception and processing of the CS, which mirrors everyday experiences.

Overall, the present findings may help to better understand the influence of learning processes on sexual behavior and its disorders. For instance, sexual (arousal) problems are not exclusively based on physiological problems [70]. The treatment of sexual dysfunction should thus be integrative [71,72]: it should combine cognitive behavior therapy (which focuses on learning processes) and medical treatments (e.g., hormonal supplementation [71,72]). Although only few empirical studies have investigated the effectiveness of therapeutic treatments (for a positive exception with many outcome measurements, [73]), Brotto et al. [74] concluded that therapeutic treatments including mindfulness practices significantly reduce sexual distress and improve different aspects of sexual arousal. One might then speculate that the increased activity in aware subjects in the ventral striatum and the insula, which is a key region for interoception and mindfulness, could mediate these therapeutic effects on the neural level. Future studies investigating neural changes caused by these therapeutic treatments should focus on these structures especially.

The greater sexual responsivity in men observed in the present study is in accordance with findings that hypersexuality and related disorders are more often found in men than in women [75]. It is also assumed that the acquisition of paraphilias and compulsive cybersex behavior arises by conditioning mechanisms, and also its therapeutic intervention is based on conditioning and extinction models [76,77]. Investigating the conditioning of sexual arousal might add valuable insights into the underlying neurobiological basis of such disorders.

In sum, we identified several brain structures involved in the acquisition of conditioning of sexual arousal. We found strong effects of awareness and sex on CRs, and highlighted the ventral striatum as a key region for conditioning of sexual arousal. Aware subjects compared to unaware subjects showed enhanced conditioned brain activation. Men showed enhanced neural activity compared to women, postulating that men are more susceptive for conditioning of sexual arousal. The presented data contribute to a more detailed insight into the neural activations of conditioning of sexual arousal.

Acknowledgments

This research was supported by the Deutsche Forschungsgemeinschaft (DFG), Research Training Group (Graduiertenkolleg), GRK 885. The authors thank Deniz Mayadali and Katrin Wistorf for their helpful support.

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Conflict of Interest: None declared.

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