2 Methods

2.1 Preparation of the experiment – pilot study

As mentioned above, the paradigm of the diploma thesis by David Meyer-Heintze (2011) showed promising results in terms of monitoring the differentiation between contextual and cue conditioning in virtual reality with electroencephalographic recordings. Due to the fact that the presented stimuli were poorly controlled, further investigations were necessary.

Moreover, in particular early event-related brain potentials (ERPs) beginning around 150 ms are highly sensitive to the properties of the eliciting stimulus (Bradley et al., 2007), so more experiments were needed comprising controlled stimulus material.
For all these reasons, a pilot study was performed to create two easily distinguishable office rooms in virtual reality that evoked equal subjective levels of pleasantness, arousal and complexity. Furthermore, for the EEG test-phase, new screenshots had to be taken and carefully chosen. Selection was done aiming for similar physical properties like brightness and entropy level. Additionally, they were supposed to subjectively appear equal in terms of pleasantness, arousal and complexity (Wessa et al., 2010).

2.1.1 New stimulus material

2.1.1.1 Rebuilding the virtual environment

In the pre-study the two existing office rooms applied in Meyer-Heintze's diploma thesis (2011) and further studies (e.g. Glotzbach-Schoon et al., 2013) were adapted using the *Hammer Editor* of the *Source Engine* from the *Valve Corporation* (Bellevue, USA). This software is mainly used in computer games like Half-Life 2, but served here as medium to adjust the original virtual environments in shape, furniture, carpet colors, view out of the windows and pictures on the walls.

The two offices were changed into quadratic shape and were connected by an elongated corridor. Since the comparability of the two rooms was essential for the main experiment, each office had to contain similar equipment and the same amount of furniture. A detailed list is shown in the supplement (A).

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In the diploma thesis (Meyer-Heintze, 2011), ERP-data reached significance just for the EPN component and that only in the office room with red compared to the room with green carpet. This color effects might be a priming mechanisms as red represents danger and green signifies safety in many everyday experiences. Literature discusses controversially if red induces avoidance motivation because it signals an upcoming danger (Mehta & Zhu, 2009) or it enhances approach behavior. From a biological point of view, red lips testify an attractive and healthy partner with high receptiveness (Elliot & Niesta, 2008). All in all, many studies showed that the color red influences human behavior (Elliot, Maier, Binser, Friedman & Pekrun, 2009). Moreover, Schupp, Junghöfer, Weike & Hamm (2003) found an explicitly increased EPN to evolutionary relevant pictures compared to neutral pictures. For this reason, the new color of the carpet in the two office rooms was chosen carefully. The original red carpet was modified in *Adobe Photoshop CS4* (version 11.0; Adobe Systems GmbH, Munich) by extending the shades of red ten steps into blue direction and ten steps into yellow direction. So two new shades of red color, a blue-red and a brown-red, were created and assigned into one of the rooms each (Fig. 2).

The view out of the window was also a prominent feature of the original virtual environment. One office room seemed to be built in a big city with skyscrapers visible in the windows. In contrast, the view out of the window of the second office revealed a hilly landscape with few one family houses. On the one hand, these different scenarios very much characterized the environments and helped the subjects to distinguish between the two rooms. But on the other hand, the differing views out of two rooms that are placed in the exact same building, appeared strange and artificial. Therefore, the newly created virtual space was set into a city. Looking out of the window, participants could now observe a similar environment from different perspectives without any distinct buildings or scenarios.

Lastly, eye-catching features in a context are pictures on the walls, which in this case occupied a very prominent space and also attracted the participants´ attention. In the pilot study, the five wall pictures in each room were selected from neutral IAPS pictures (International Affective Picture System; pictures number 1121, 5390, 5395, 7160, 7247, 7248, 7249, 7547, 7820, 7830; Lang, Bradley & Cuthbert, 1999) according to a medium valence and arousal level and then equally distributed into the rooms

(valence: $t(4) = -1.463$, $p = .217$; arousal: $t(4) = .462$, $p = .668$). All these modifications of the virtual environment should improve the comparability of the stimulus material in the main experiment.

Furthermore, in the pilot study paths were recorded through each office room, starting in the corridor. They lasted for 110 s in one and 111 s in the other room, respectively, and ended again in the corridor. Figure 2 depicts the two new office rooms right after entering one room.

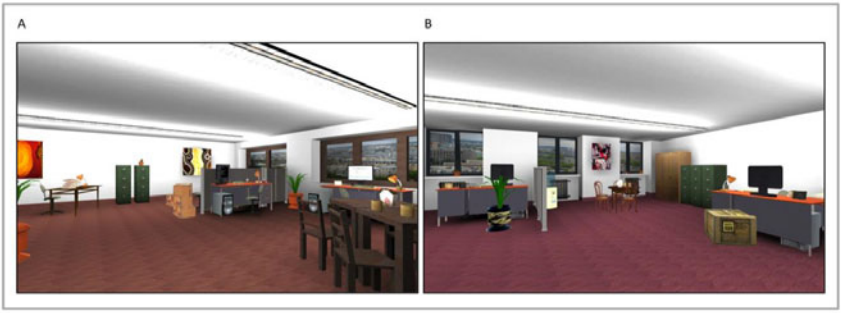

Figure 2: The two newly created office rooms. 2A depicts the room with brown-red carpet and 2B represents the room with blue-red carpet.

2.1.1.2 New screenshots

As a next step, screenshots were taken of the virtual environment from which pictures could be selected serving as stimuli in the test-phase. These pictures of the office contexts were presented in the main experiment to evoke event-related brain potentials. For that reason, starting the pre-recorded path through the offices, screenshots of the virtual rooms were taken every 2 seconds. Excluding pictures of the corridor, 42 potential stimuli in each room were created for the EEG experiment. To control for physical properties, entropy levels were calculated in *MATLAB* (version 7.11.1, MathWorks, USA). The mean entropy values of all 42 screenshots did not differ between the two rooms, $t(41) = .477$, $p = .636$. Moreover, RGB-values comparing the brightness of the pictures of both rooms were determined applying *Adobe Photoshop CS4* (version

11.0, Munich, Germany). It could be shown that the created stimuli of the two rooms did not differ in brightness, $t(41) = -1.357$, $p = .182$.

2.1.2 Evaluation of the stimulus material

2.1.2.1 Sample and procedure

In order to investigate the subjective perception of the screenshots, 14 participants (8 females; mean age: 24.7; *SD* = 3.97) took part in the pilot study. They observed the virtual environment on the powerwall using *Cybersession* software (version 5.3.38). Participants were passively guided via the pre-recorded paths through the two newly created office rooms. After that, subjects were asked to rate which office seemed more pleasant. Additionally, participants saw each screenshot and were asked to rate their subjective feelings of valence, arousal and picture complexity directly after observing each picture individually. The rating experiment was created with *Presentation* software (version 15.1, Neurobehavioral Systems, Inc.).

2.1.2.2 Results and conclusions

Results showed that seven subjects preferred the room with the red-brown carpet color (Fig. 2A) and six the other office with red-blue (Fig. 2B), one participant could not decide, so he rated both rooms equally pleasant. Comparing these results, χ^2-test revealed that the overall valence of both offices was on the same level, $\chi^2(1) = .076$, $p = .783$.

Data suggest that for the conditioning procedure, the newly created virtual contexts did not differ before conditioning. Therefore, any significant effect during conditioning could be ascribed to the experimental manipulation instead of virtual reality effects. Besides, screenshot ratings of both offices neither differed in valence, $t(41) = 1.704$; $p = .096$, nor in arousal, $t(41) = -.384$; $p = .703$, and picture complexity, $t(41) = -.278$; $p = .783$.

Now, the most suitable screenshots of each office room were chosen for the experiment that showed similar physical picture properties and comparable subjective ratings of valence, arousal and complexity. Therefore, 24 out of 42 stimuli per room

were selected and analyzed concerning all described parameters. Mean values and *t*-test statistics are depicted in Table 1 and clarify that the screenshots of both rooms do not differ significantly in the listed objective and subjective properties. Room 1 refers to the office with brown-red carpet and room 2 to the office with blue-red carpet.

Table 1: Objective and subjective picture properties of the 24 selected screenshots of each office room, mean values (*M*), standard deviation (*SD*) and t-tests.

	room 1		*room 2*		*statistics*	
	M	*SD*	*M*	*SD*	*T*	*p*
entropy	70972.33	4225.47	70083.83	2542.32	0.877	n.s.
RGB-value	154.70	7.12	157.96	6.78	-1.50	n.s.
valence	5.30	0.53	5.25	0.47	0.315	n.s.
arousal	4.65	0.29	4.68	0.28	-0.447	n.s.
complexity	5.85	0.97	5.79	0.88	0.261	n.s.

t-tests: $p > .05$: not significant (n.s.)

Furthermore, the 24 pictures were divided into two groups, which were presented during the experiment with or without US, respectively. Focus lay on the time interval, which was at least 4 s between two consecutive stimuli of different conditions in one room. In total eight experimental procedures were designed to pseudo-randomize the order of the two rooms in the acquisition phase, the anxiety (CXT+) and the safety context (CXT-) and the screenshot group which was associated either with (CXT+US) or without US (CXT+noUS) in the anxiety context.

In sum, the screenshots that were investigated in the pilot study should now be well adapted for the main study.

2.2 Sample

Participants were recruited via the local online platform *wuewowas.de*. At first a pre-screening by phone was conducted to exclude psychology students and people with excessive alcohol or illegal drug consume, regular intake of centrally active medication, severe illnesses or psychological disorders, poor eyesight, color blindness, left-

handedness and pre-experience in similar conditioning experiments in virtual reality (supplement C).

In total 32 subjects took part in the experiment. Four unaware participants were excluded from further data evaluation because they obviously were not able to explicitly recall the CXT+US contingency. Therefore 28 subjects (13 females) aged between 18 and 33 (M = 23.81, SD = 3.98) were included in data analysis.

Participants were invited to the powerwall laboratory where they perceived a detailed written explanation about the experimental procedure. The study was approved by the Ethics Committee of the Medical Faculty of the University of Würzburg. The experiment lasted about two hours and was generally refunded with 16 Euros.

2.3 Material

2.3.1 Stimulus material

2.3.1.1 Aversive electric stimulation

In order to elicit fear, a mildly painful electric stimulus served as an unconditioned stimulus (US) in the experiment, administered by a *Digitimer Constant Current Stimulator Model DS7A* (Digitimer Ltd, Hertfordshire, England). The US consisted of a 50 Hz pulse, which lasted 200 ms (Andreatta, Mühlberger, Yarali, Gerber & Pauli, 2010). Before the pre-acquisition phase started, each subject's individual pain threshold was determined. Therefore, the electrode was placed at the participants' right inner forearm. Several electric stimuli were administered. Subjects rated their feeling of pain on a scale from 0 (*no sensation at all*) until 10 (*unbearable pain*) directly after each shock. Especially highlighted was score 4, because this estimation should be chosen when the stimulus elicited *just perceptible pain*. The current of the electric stimuli was increased in 0.5 mA steps, starting with 0 mA, until the participant rated a stimulus on the scale above 4. Then a descending series of electric stimuli was initiated, also in 0.5 mA steps until the subject scored the electric stimulus below 4. This procedure was repeated twice. Then, the individual stimulus intensity was calculated by averaging the four current intensities that were rated by at least 4. This intensity was increased by 30% to assure an aversive, moderate pain feeling for the subjects and to avoid habituation. This

stimulus was administered to the participant and once again rated on the 10-point pain scale. The shock was regarded as well adapted, when the score was above 4 and the subject affirmed his or her agreement to deal with the pain in the experiment. Moreover, valence and arousal ratings of the electric stimulus were requested. The valence scale ranged from 0 (*extremely negative*) to 100 (*extremely positive*) and the arousal scale from 0 (*not arousing at all*) to 100 (*extremely arousing*) (supplement G).

2.3.1.2 Screenshots in test-phase

In the test-phase of the experiment, screenshots depicting the virtual office rooms were shown for 1000 ms to evoke event-related potentials in the cortex of the brain. Inter-stimulus intervals varied between 1500 and 2500 ms. The screenshots were carefully chosen according to their physical properties and subjective ratings developed in the pilot study mentioned above. Depending on the experimental condition, 24 screenshots of the safety context (CXT-) and 24 of the anxiety context (12 CXT+US; 12 CXT+noUS) were presented three times, so the EEG experiment comprised a total number of 144 trials. As already explained above, safety and anxiety contexts as well as groups of screenshots with and without electric shock were counterbalanced across participants.

2.3.2 Data acquisition

2.3.2.1 Subjective data

Data about the subjects' personal mood and emotional state were collected using standardized questionnaires at the beginning of the study and ratings integrated into the experimental procedure.

2.3.2.1.1 Questionnaires

Before starting the conditioning experiment, participants were provided with questionnaires, which were rendered anonymously. In general, questionnaires are a good option for investigating not only the subject's demographic data, but also very subjective feelings, as e.g. Jackson, Payne, Nadel & Jacobs (2005) showed, that stress

modulates classical fear conditioning.

First, demographic information (age, gender, education, profession and handedness) was collected. Moreover, inclusion criteria were requested another time in written form, especially in regard to the subject's liability to nausea (supplement F). This is an inevitable aspect to control in virtual environment studies because susceptible people often reported a feeling of sickness when moving through virtual reality (Bohil et al., 2011).

The Positive and Negative Affect Schedule (PANAS; Watson, Clark & Tellegen, 1988; German version by Krohne, Egloff, Kohlmann & Tausch, 1996) determines the subject's momentarily emotional state in dominant two dimensions. The positive affect part describes the extent of a person's enthusiasm, activity, attention, lethargy and sadness, whereas the negative affect part gathers the degree of negative tension, petulance, nervousness and anxiety. The questionnaire consists of two mood scales containing ten items each, to investigate positive and negative affect.

Moreover, the subjects' fear of fear was investigated by the anxiety sensitivity index (ASI; Reiss, Peterson, Gursky & McNally, 1986; German version by Alpers & Pauli, 2001). In this questionnaire, authors examined anxiety sensitivity and focused negative implications that were associated with anxiety experiences. Here, 16 items request the subjects' anxiety. It can be answered in a five-point scale depending on the participant's own estimations.

The Behavioral Inhibition System and Behavioral Approach System questionnaire (BIS/BAS; Carver & White, 1994; German version by Strobel, Beauducel, Debener & Brocke, 2001) is constructed in 24 items, which can be rated by the subjects on a four-point scale. The questionnaire gives indications of the subject's sensitivity to reward and punishment. Therefore, the BIS is responsive for cues of punishment and a measure of vulnerability to anxiety. The BAS, in contrast, is sensitive to cues of reward including the investigation of a subject's fun seeking and drive.

Finally, the State-Trait-Anxiety-Inventory (Spielberger, Gorsuch & Lushene, 1970; German version by Laux, Glanzmann, Schaffner & Spielberger, 1981) examined the participants' current and general anxiety. This questionnaire differentiated anxiety in two kinds. First, anxiety is seen as an emotional state, which is characterized by tension, apprehensiveness, nervousness and increased activity of the autonomic nerve system.

Second, trait anxiety is investigated which refers to subjects' estimations of a situation as threatening. Stable inter-individual differences become evident in a way that high anxious individuals tend to classify more situations as dangerous compared to subjects with low trait anxiety. The STAI-state as well as the STAI-trait part each consists of 20 items. In both parts, participants were asked to rate on a four-point scale.

2.3.2.1.2 Explicit ratings: Valence, arousal, anxiety, US-expectancy, US-contingency and screenshot affiliation

The experiment consisted of several phases in which subjective ratings were used as dependent variable. Levels of the participants' valence, arousal and anxiety were asked three times during the conditioning: directly after pre-acquisition, after acquisition phase 1 and after acquisition phase 2 (see Fig. 3). Objective of these ratings was not only the investigation of the participant's feeling but also the monitoring of gradual changes of their feelings during the conditioning paradigm. Rating was conducted in the following way: An instruction slide was shown on the powerwall that described the procedure and the actions that were required of the participant. Afterwards, a screenshot serving as a reminder of the context depicting the entrance of one of the office rooms was shown. Meanwhile, a question popped up concerning the feeling when the subject was guided through the depicted room, requesting valence, arousal or anxiety estimations respectively. Answers could be given on a scale from 0 to 100. In the valence rating, 0 meant a *very negative feeling*, 100 implied a *very positive feeling* in the distinct office room. The range of the arousal rating also was from 0, *not arousing at all*, until 100, *extremely arousing*. Anxiety levels could be scaled from 0, *no anxiety at all*, until 100, *extreme anxiety*. Then, this procedure was repeated for the second office room. All ratings were orally reported by the subject and noted by the experimenter. At the end of rating phase three, another rating slide was added requesting the US expectancy in the two rooms, respectively. Another scale ranging from 0 till 100 was shown, 0 representing *no expectation* of an electric shock and 100 representing *complete expectation* of US. Screenshots applied in this rating only served as labeling of the concerning context and were not used in the test-phase (supplement J).

After the EEG test-phase, participants saw each screenshot once again (12 CXT+US, 12 CXT+noUS and 24 CXT-) for 1000 ms and were asked whether a US was presented in the acquisition phase exactly at the time depicted in the screenshot. This part of the experiment was conducted with *Presentation* software (version 15.1, Neurobehavioral Systems, Inc.). Subjects typed their ratings on a 100-point scale into the computer by using a keyboard. 0 signified that *surely no US was presented* and 100 implied that participants *surely remembered an electric shock* at this position (supplement L).

The participants' last task was to assign each screenshot to one of the office rooms. Therefore, screenshots were presented again for 3000 ms with *Presentation* software (version 15.1, Neurobehavioral Systems, Inc.) and participants were asked to allocate the pictures to the context in which they were taken (supplement M).

2.3.2.2 Physiological recordings

Skin conductance level and electroencephalogram were recorded as objective and psychophysiological indicators of induced fear and anxiety.

2.3.2.2.1 Skin conductance level

During the acquisition phases, physiological reactions were measured by the subjects' skin conductance level. Measurements were taken by filling two 13/7 mm Ag-AgCl electrodes with 0.5% NaCl-paste and placing them on the thenar eminence and hypothenar eminence of the left non-dominant hand. Data collection was extended over pre-acquisition and acquisition phases 1 and 2, starting when a subject entered a room in virtual reality and ending when the participant was guided out of the room and into the corridor. Data was recorded via the software *Vision Recorder* (Brain Products GmbH, Munich, Germany) on a mobile notebook.

2.3.2.2.2 Electroencephalography

Event-related brain potentials (ERPs) were measured by applying an international 10-20 EEG system (Acticap, Brain Products GmbH, Munich, Germany). This 32-channel

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