Research Article

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Contextual pain conditioning protocol design in Humans – a combination of behavioral and physiological measurements using immersive Virtual Reality

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ABSTRACT

Contextual pain conditioning is the process of associating a specific painful experience to its emotional and environmental context. Although pain conditioning is suggested to play a key role in the development and maintenance of chronic pain, there is still paucity of studies and protocols specifically devoted to better understanding its underlying mechanisms. The present study offers an ecological experimental model of contextual aversive (painful and non-painful) conditioning using immersive virtual reality (iVR) technology. The protocol comprised both context and sensory stimuli, specifically: i) a three-roomed virtual apartment presented in iVR, ii) a painful aversive electro-stimulation (PA), and iii) non-painful aversive sounds (NPA). Acquisition and extinction phases consisted in participants completing ten rounds in the apartment, for which each room was associated to a specific stimulation delivery (PA, NPA or no stimulation for control). Sympathetic skin response (SSR), environment valence perception and stimulation evaluation were measured throughout the entire experiment to assess behavioral and physiological markers of aversive conditioning. Results confirmed that both aversive conditionings lead to a decrease in associated room valence compared to control, even more so for the PA associated room, which resettled after extinction. Together with a significantly greater SSR found during anticipation of pain in contrast to both aversive sounds and control, these outcomes may suggest that pain conditioning acquisition was stronger than non-painful aversive conditioning. Concluding modulation of aversive conditioning markers enabled to validate this protocol, which future application may be adjusted to allow studies in chronic pain patients.

INTRODUCTION

Being subject to pain in association with a specific event is suggested to facilitate the induction and maintenance of chronic pain through classical conditioning (Faasse and Johnson 2008, Madden et al. 2016, Moseley and Vlaeyen 2015, Vlaeyen, 2015, Koenig et al. 2021). Chronic pain can be described as a state of persistent learning in which the continuous presence of pain generates constant aversive associations with daily events (Apkarian et al. 2009, Mazza et al. 2018, Harvie et al. 2017) resulting in pain-related fear conditioning. Though pain-related fear conditioning can in some cases be cue-specific, where patients report knowledge of pain outbreaks in the presence of specific stimuli (Johnson et al. 2006), non-cue specific pain occurrences can also lead to contextual pain-related fear conditioning (Meulder 2020, Meulders et al. 2011, 2013). Indeed, research has shown that an unpredictable painful unconditioned stimulus (US) applied in the absence of distinguishable cues causes the context in which it is delivered to be perceived as a potential hazard and leads to continuous anticipation of threat (Meulders et al. 2011; Fonteyne et al. 2010; Vansteenwegen et al. 2008). Although contextual pain-related fear has been reported to play a key role in the persistence of chronic pain (Keefe et al. 2004; Meulders 2020; Mazza et al. 2018), we noted a lack of research concerning experimental models aimed at a better understanding of its mechanisms.

A comprehensive literature of contextual fear conditioning is available, specifically in animal studies (Yu et al. 2021, Schroyens et al. 2019, Kenney et al. 2017, Curzon et al. 2009), where context stimuli are easily constructed and modelled

Journal of Pain (ISSN 2996-1793)

under experimental requirements by simply modifying the environment's properties (e.g. different spaces changing color, texture or odor) or changing the animal's location. In comparison, contextual fear conditioning studies in humans struggle to meet such criteria, as it would analogically require subjects to physically move from one location to another , i.e. require settings that are unavailable in experimental laboratories. In recent years, we noted an increasing number of studies aiming to bridge the gap between animal and human research by using innovative and ecological working-tools such as immersive Virtual Reality (iVR) (Kroes et al. 2017, Houtemaker et al. 2020, Reichenberger et al. 2020, Binder and Spoormarker 2020). The virtual but very vivid experience induced by iVR entails the illusion of presence, expressed as the combination of physical and emotional belonging to the virtual world in which our actions have repercussions (Botella et al. 1998, Lombard and Ditton 1997, Riva et al. 2015).

iVR has been shown to optimize context stimuli and enhance emotional implication during contextual learning (Kim et al. 2014, Kroes et al. 2017), by virtue of an effective "sense of presence", whereby users report the illusion of being in a virtual environment in which occurrences truly happen (Sanchez-Vives et al. 2005). In 2010, the Duke University virtual environment (DiVE) system initially introduced immersive context at the profit of contextual fear conditioning based on a projected virtual reality "CAVE" design (Cruz-Neira et al. 1993) described as a fully enclosed 30 feet cube-shaped room in which the virtual environment is projected on all 4 walls, ceiling and floor (Huff et al. 2010). Nowadays, iVR contexts are widely displayed through immersive Head Mounted Displays (HMDs) (Kroes et al. 2017). A commercially accessible iVR environment comprising a 2-separate-room area connected by a hallway, combined with an unpleasant stimulus (electric shocks), resulted in an effective threat conditioning protocol (Kroes et al. 2017). This system was further used as a context tool for threat conditioning studies, investigating the effect of a reminder prior to extinction on contextual mnemonic performance, physiological reactivity (skin conductance, eye blink, heart rate) and behavioral measures (context perception, stimulus rating) (Houtekamer et al. 2020), as well as other similar tool systems investigating specifically conditioning-related avoidance behaviors (Reichenberger et al. 2020, Binder and Spoormarker 2020). However, we noted that such laboratory studies often resort to pre-fabricated items or even entire environments available cost-free on the cross-game platform engine Unity (Unity Technologies, www.unity3D.com) typically used to establish VEs. Although very efficient, these elements remain visually basic and stereotyped, resulting in a cleavage with the outside real world which possibly deteriorates the quality of immersion. Indeed, the quality of VR experience relies on the correspondence between the visual stimuli and the level of expectation of the user (Baños et al. 2000): the higher is the similarity between the virtual stimulus and real-life stimulus, the closer to reality is the outcome of the user's behavior. Considering that most studies focus on behavioral measurements, tackling this issue could contribute to better iVR systems and therefore improvement in experimental research.

Our goal in this project was to contribute to contextual pain conditioning research by developing new experimental models using innovative techniques. Here we offer a complex contextual aversive (painful and non-painful) conditioning protocol elaborated using an ecological environment modelled in iVR. The virtual environment has been fabricated from the ground up, and every single item was modeled so to ally realism, practicality and immersion quality in experimental settings. The protocol aims to offer a comprehensive view of contextual conditioning processes as it combines both behavioral and electrophysiological measurements. This paper thoroughly describes the construction of the protocol.

METHODS

Context stimulation

iVR equipment : The iVR system used was the HTC VIVE technology which comprises VR headset, cameras called base stations and a set of controllers. VR headset display offers a 360° field of vision (120° horizontal, in a stable position), with a refreshing framerate at 120Hz, which translates into a smooth display of movement. Pixel resolution goes up to 2448x2448 pixels per eye-screen. HTC VIVE headset and controllers are compatible with SteamVR® 1.0 and 2.0 base stations which detect the headset position (user's position) and configurate the field of movement available. Cameras are placed in all corners of the room to define the movement space range: 10m x10m range of movement with four base stations and 5mx5m range with two stations.

Virtual context : The context stimulus is a 3D-apartment modelled in immersive Virtual Reality using Blender2.92 ® and Unity 2018.2.16f1 ® software (Unity technology, www.unity3d.com). The apartment is composed of a kitchen, a living-room and a bathroom, connected via a central hallway (figure 1A). The three-roomed architecture was chosen to create three neutral contextual stimuli, each to be associated with one specific condition: painful aversive condition (PA), non-painful aversive condition (NPA) and control condition (CONT). An avatar was designed to match specifically the user's position and seating (figure 1C). C# scripts were used to define the avatars movement around the apartment, the incidental encoding task described in part 2.4 and room rotation around the hallway allowing randomized context stimuli appearance (Figure 1B).

Supplementary data is available on an Open-source platform: https://github.com/argitxuCaldichoury/VEProtocol.git.

Journal of Pain (ISSN 2996-1793)

Specifically, the data includes: i) an explanatory document which details the implementation of the protocol, ii) the code used to implement the experimental flow and iii) a video illustrating a circuit around the virtual environment.

Participants

This study was approved by a National Ethics Committee (Comité de Protection de Personnes CPP Nord-Ouest IV n° 2019-A01816-51, ClinicalTrials.gov ID: NCT04189965) and declared at the Liberties and Informatic National Commission (CNIL). Participants were recruited through announcements at the Neuroscience Research Center (CRNL) and universities of Lyon, signed informed consent, and were remunerated for their participation. Were excluded subjects suffering from any type of chronic pain or under drug treatment with analgesics, subjects suffering from any psychiatric disorder and subjects with heart abnormalities and/or abnormal long-term mnemonic capacities (tested at the beginning of the experience with The Doors and People memory Test (D&P; Baddeley et al., 1994)). Sixty volunteers participated to the final version of the experiment, out of which 1 was discarded due to an exclusion criterion revealed at the end of the experiment; hence, n=59 healthy volunteers participated to the final study (25.77± 5.5 y.o; 29 women). No subjects of the final experiment were excluded for iVR intolerance nor aversive stimuli intolerance. Based on previous research of skin conductance effects in a contextual aversive iVR conditioning protocol reporting an effect size of 0.73 (Kroes et al. 2017) with a sample size of 22 and a power of 95%, error α=0.05 (Gpower 3.1.9.2 , we estimated that a sample size n=59 subjects allows to disclose a difference in physiological markers analysis between the before and after conditioning phase.

Sensory stimulations

Nociceptive stimulation

The nociceptive stimulation was an electric stimulus distributed homogeneously on the left hand's surface creating a feeling of paresthesia mainly on the palm of the hand. It was though an Electrostimulation Glove (Axion®, France) acting as cathode, and a 5*5-centimeter plane electrode placed on the wrist (anode), and driven by a Micromed stimulator (Micromed®, France) with the following parameters: frequency 20 Hz; impulse duration 1000µs; 800 impulse iterations (**Figure 1**). The stimulus lasted 40 seconds and its intensity was determined for each subject at the beginning of the experiment by using a scale rated from 0 to 10 where 0 was defined as 'non-painful', 10 as 'Extremely painful' and 4 being the nociceptive threshold. After calibration, the final chosen stimulus intensity had to match between 5 and 6 on the rating scale, which means a stimulation felt as painful but tolerable. The current intensity varied between 12mA to 28mA throughout all participants: for women, the average current intensity was 15+/-4.8mA; for men 17+/-4.8mA. During the experiment, participants systematically rated verbally the painful stimulation, allowing to adjust the current intensity in case of habituation (ratings below 5 out of 10 on the rating scale) or sensitization (ratings above 7 out of 10). Adjustments varied around ±4mA around the initial calibration. The benefit of using an electro-stimulation glove is the wider surface of stimulation it offers compared to simple electrodes, which in turn allows a better modelling of the pain described in clinical settings (Peyron et al. 1998).

Figure 1

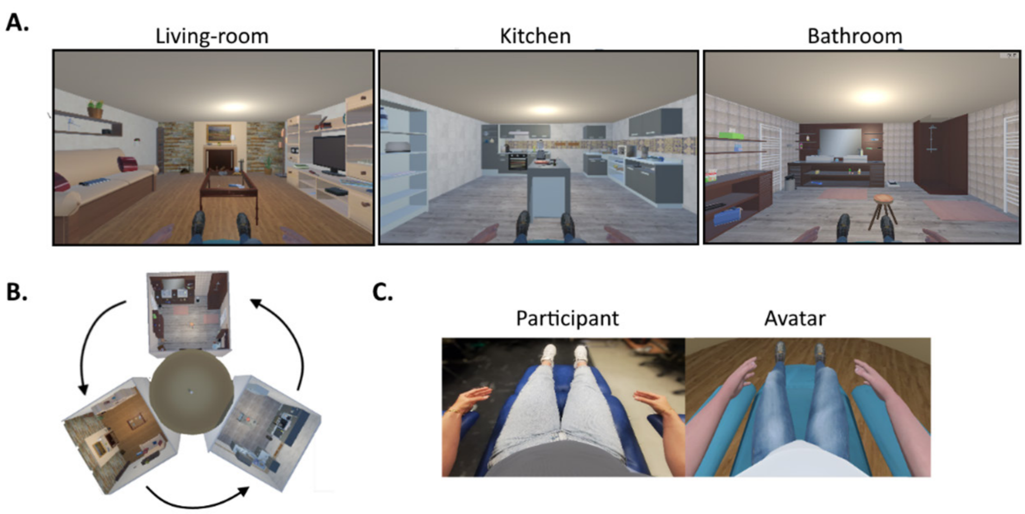

Figure 1: *Virtual environment A. Apartment composed of a living-room, a kitchen and a bathroom. B. Apartment structure allowing room rotation around the central hallway, C. Virtual avatar imitating participants' position and location.*

Journal of Pain (ISSN 2996-1793)

Non-nociceptive aversive stimulation

The non-nociceptive aversive stimulation was based on auditory unpleasant stimuli delivered through Bluetooth headphones (Sonic.), with a volume set on 67dB. The volume allowed clear but painless hearing of aversive sounds. The stimulus was a set of seven mixed soundtracks each composed of three aversive sounds (supplementary data).

Seven different categories of sounds were used (knife rubbing against glass bottle, bike brakes, street drill, chalk against board squeaking, door squeaking, fork rubbing on plate, nail scratching), each recorded in various exemplars giving a total of 60 sound samples of 6 to 10 seconds duration.

The samples were assessed via a questionnaire uploaded online to evaluate the following features: (i) averseness, using a 10-point scale 1 corresponding to 'Not aversive' rating and 10 to 'Extremely Aversive', and (ii) relation to pain, evaluated with a choice-answer question: "How much does this sound remind you of physical pain?". Possible answers included: "not at all", "slightly", "moderately", "a lot", "extremely". Answers were translated into numerical values going from 1 to 5 points. This latter scale was required as our goal was to have an aversive stimulation completely separated from pain or any pain-associations. Sound samples are available on request.

Sixty-one volunteers answered the questionnaire (37.42 ± 16.4y.o; 31 women). Results enabled to select sounds according to the following criteria: a minimum mean score of 5 out of 10 on the aversion scale, and below 2 out of 5 on its relation to pain. Twenty-one sounds were by means included and divided in seven groups composed of 3 sounds with equivalent mean aversion (5,8±0,06) and mean association to pain (1,6±0,04) resulting in seven mixed soundtracks.

Data acquisition

Immersion evaluation

For immersion quality testing, participants were required to evaluate their iVR experience in terms of location and presence in the environment ((ii) and (iv)) and body ownership ((i) and (iii)). Subjects were asked to indicate whether or not they agreed with the following affirmations: (i) "My (real) body started to embody the posture or form of the virtual avatar" (Posture) (ii)" My (real) body could be affected by the environment" (Possibly affected) (iii) "The avatar's body was my own" and (iv)" My (real) body was placed in the same location as the avatar's body". Seven available answers were given: 1. Never 2. Rarely 3. Occasionally 4. Half of the time 5. Often 6. Almost always 7. Always. Theses answers were then translated into a 6 point-scale, 0-points being "Never" and 6-points being "Always". Descriptive analysis of immersion quality was done by averaging numerical answers across all subjects for each affirmation.

Behavioral Tests

Behavioral measurements were programmed on PsychoPy®. For our protocol, we programmed two behavioral measurements : environment evaluation and stimulation evaluation (figure 2B):

(i) Environment evaluation: Subject's perception of the environment was measured via the valence attributed to each room using the Self-Assessment Manikin Valence Scale (Bradley & Lang, 1994).

(ii) Stimulation evaluation: Painful and non-painful aversive stimuli were assessed with Visual Analog Scales (VAS). For the painful stimulation, subjects rated both the level of unpleasantness and the pain intensity of the electro-stimulus: the VAS for the level of unpleasantness ranged from "Not unpleasant" to "Extremely unpleasant"; the VAS for pain intensity ratings ranged from 'Not painful" to "Extremely painful". As for aversive sounds, subjects evaluated the level of unpleasantness only (same VAS described above).

Sympathetic skin response (SSR)

The SSR has been extensively used as an indicator for variations occurring in the autonomic nervous system during the establishment of a conditioned response (Bräsher and Witthöft 2019, Faghih et al. 2015, Esteves et al. 1994, Sjourwerman et al. 2015). Sympathetic skin response was recorded continuously using the acquisition system ASA (ANT Software, The Netherlands). One electrode was placed on the palm of the right hand and another on the opposite side as reference-electrode.

Experimental Procedure

Participants were comfortably seated in a lounge chair either in front of a screen during testing or wearing the Head Mounted Display HTC Vive ® during immersion. The experiment comprised three main periods: familiarization, conditioning and extinction period (Figure 2A).

Familiarization

Participants had to first and foremost adjust with iVR. Familiarization consisted in immersing the participant in the virtual apartment for one single round around each room, thus lasting less than 4 minutes in total. The subject was instructed to stay still on the chair and move the head only slightly from right to left, up and down, and avoid any brusque movement. This procedure enabled both: (i) to validate the participant's tolerance towards immersion and confirm their ability to proceed with the rest of the experiment and (ii) for the subject to discover all three rooms prior any type of conditioning, as neutral stimulations. After familiarization, subjects were asked to evaluate their perception of the environment, i.e. measure the valence and emotional impact of each room pre-conditioning.

Conditioning

The conditioning phase consisted in creating two types of aversive conditioning (PA and NPA) to be compared to a control condition (CONT). To do so, each room (kitchen, living room, bathroom) was randomly attributed to a specific condition (painful aversive (PA), non-painful aversive (NPA) or control (CONT)), thus variating from subject to subject. Participants were immersed for 35 minutes in the virtual apartment, during which they proceeded to complete ten rounds in each room. Participants stayed 49 seconds in each room with a 25 second stay in the hallway in between each room visit. According to the room they entered, the experimenter either induced the electro-stimulation (PA condition), the aversive sounds (NPA condition), or no stimulation at all (CONT condition). Stimulations were delivered between 1 to 5 seconds during room entrance. In the aversive rooms, for three rounds out of ten, no stimulations were delivered, these being randomly distributed across all ten rounds for each condition (the electro-stimulation free rounds and sound-free rounds not being necessarily simultaneous). This enabled to monitor the development of both conditionings throughout the entire phase.

Each time subjects exited the PA-room (where the electro-stimulation is delivered), they were asked to rate the pain intensity and unpleasantness of the stimulus they had just felt. After conditioning, subjects were asked to evaluate the valence and emotional impact of each room post-conditioning, rate the average pain intensity and pain unpleasantness of all seven electro-stimulations received and rate the mean unpleasantness of all seven aversive soundtrack stimuli (figure 2B).

Figure 2

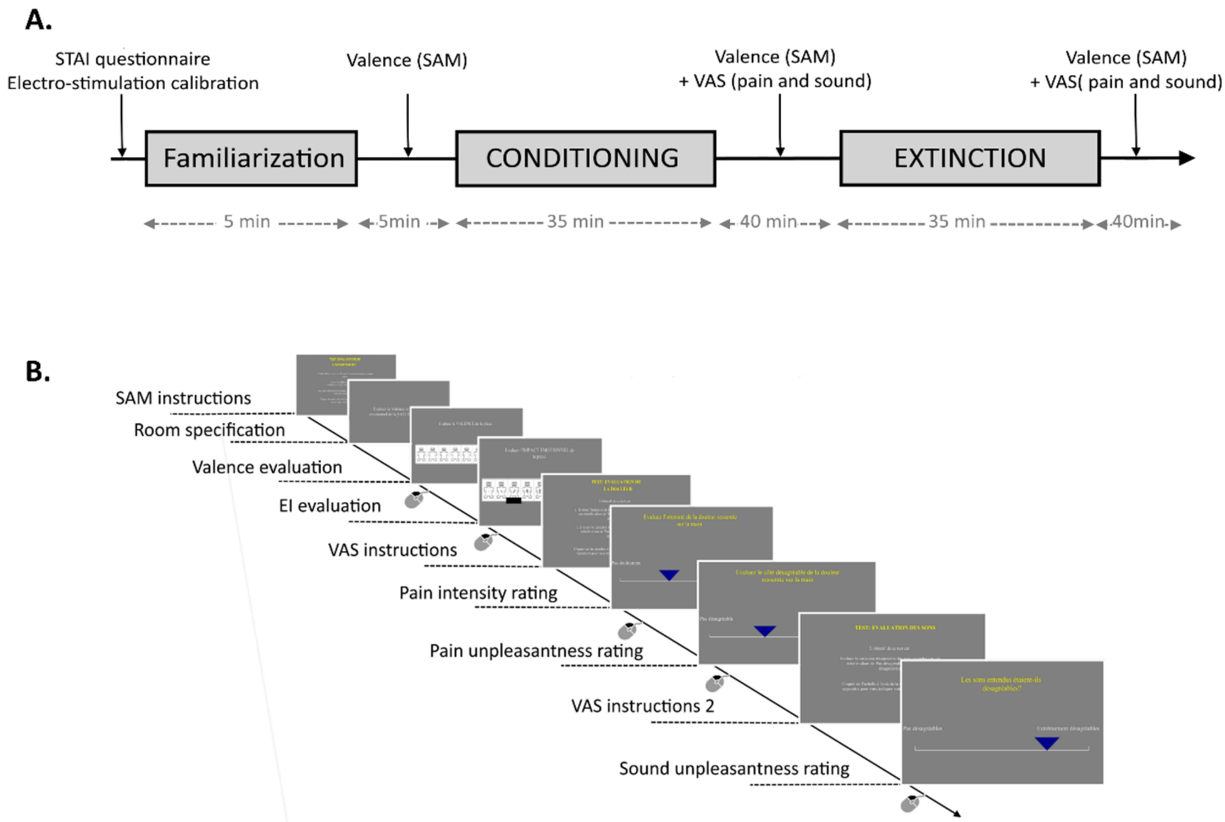

Figure 2 : *A. Experimental protocol timeline : familiarization, conditioning and extinction session with behavioral measurements (Valence and EI, VAS, Implicit memory testing) in between each phase; STAI : State and Trait Anxiety Inventory ; EI : Emotional Impact ; VAS : Visual Analog Scale ; PA : Painful Aversive ; NPA : Non-Painful Aversive. B. Behavioral testing sequence : Valence and Emotional Impact (EI) evaluation (SAM scale) for each room (kitchen, living-room; bathroom) followed by Pain intensity and Pain unpleasantness ratings (VAS) and Sound unpleasantness rating (VAS).*

Extinction

Three groups of subjects were defined for extinction: (i) a **Total-extinction** group: both aversive conditionings were extinguished, the procedure was exactly the same as in conditioning phase with no stimulation delivered, (ii) a **PA-extinction** group: only the painful aversive conditioning was extinguished, aversive sounds were again delivered and (iii) an **NPA-extinction** group: only the non-painful aversive conditioning was extinguished, electrostimulations were again delivered. These different groups of extinction allowed to analyze the long-term effects of extinction particularly on behavioral measurements, e.g. environment perception and stimulation evaluation.

Data analyses

All statistical analyses were performed with JASP® software with significance level set at $p<0.05$, and Greenhouse-Geisser correction applied to repeated-measures ANOVA when needed. Holm post-hoc corrections were used when necessary. In all the figures, data are presented as mean ± SEM.

Behavioral data

Stimulations perception: Intensity ratings were measured post-conditioning for the painful stimulation only. Unpleasantness ratings were measured post-conditioning for the painful electro-stimulation (Pain), and for the non-painful aversive sounds (Sound). A Student-t test was performed between stimulations (pain and sound) unpleasantness ratings.

Environment perception: Valence of each room were assessed at three different time-points for every subject: after familiarization (Pre-COND), after conditioning (Post-COND), and after extinction (Post-EXT). A repeated - measures ANOVA was done using time point (PreCOND, PostCOND, PostEXT) and room (PA, NPA, CONT) as intra-subject factors.

Physiological data

Data pre-processing: SSR recordings were pre-processed on The Observer XT © software (Noldus - Wageningen, the Netherlands). This behavioural research tool software enables to synchronize and analyse multiple inputs (e.g. video, audio, physiological signals, etc.) of an experiment. In our case, it was used to synchronize the timeline of the experiment using a recorded video of the virtual environment conditioning circuit and the recorded physiological data. This allowed to extract specific segments of the SSR recorded data according to the subject's location (PA, NPA, CONT or hallway) and stimulation delivery (either ON or OFF according to the round).

To alleviate further writing, SSR of the PA condition-associated room location will be referred as PAON (when the electrostimulation is ON), PAOFF (for when it is OFF); as for the NPA condition associated room location (NPAON, NPAOFF). The control condition associated room location will be referred as CONT and the hallway location serving as baseline will be referred as BL.

For the conditioning phase, the following SSR segments were exported for each subject: (i) seven segments PAON, seven segments NPAON, three segments PAOFF, three segments NPAOFF, ten segments CONT – all 49 seconds long each (duration of the subject's presence in a specific room), and thirty BL segments - 25 seconds long.

For the extinction phase, the number of ON/OFF segments varied across subjects according to their specific extinction-group-belonging (Total extinction, NPA extinction, PA extinction).

- For the total extinction groups ten segments PAOFF, ten segments NPAOFF, ten segments CONT and thirty segments BL were extracted.
- For the NPA extinction groups: ten segments NPAOFF, ten segments CONT and thirty segments BL.
- For the PA extinction groups: ten segments PAOFF, ten segments CONT and thirty segments BL.

All segments of every condition (PAON, PAOFF, NPAON, NPAOFF and CONT) and baseline segments were rectified before further processing.

Conditioning phase analysis: All different types of SSR segments were averaged within their condition so to finally obtain six averaged SSR segments in total for each subject: PAON, NPAON, PAOFF, NPAOFF, CONT. To enable baseline correction, 15 artificial segments of 49 seconds were created out of the initial 25 seconds long BL segments (see example Figure 3). Baseline segments were then averaged to create a single 49 seconds long BL segment for each subject to match the length of each condition segment.

To eliminate non-specific effects, the average baseline was subtracted to each condition: PAON - BL, NPAON - BL, PAOFF - BL, NPAOFF - BL, CONT- BL. To alleviate writing these will be further referred as: PAON, NPAON, PAOFF, NPAOFF, CONT.

Averaged segments were divided into four 10 seconds-long intervals starting at 3s after event onset to avoid any contaminations (e.g. the shock of the corresponding stimulation or associated startle probe), intervals will be referred as: 0-10s, 10-20s, 20-30s, 30-40s). This allowed to investigate the evolution of SSR throughout the entire duration of a specific room (see example Figure 4). For each interval of each segment, amplitude of the SSR was measured peak-to-peak of the largest deflection of the corresponding interval, in keeping with previous studies (Chapon et al. 2019, Kroes et al. 2017).

A two-way repeated measures ANOVA was performed with conditions (PAON, NPAON, PAOFF, NPAOFF, CONT) and time intervals (0-10 s, 10-20 s, 20-30s, 30-40s) as inner-subject factors, with Greenhouse-Geisser correction. Holm-corrected post-hoc t-tests were added when necessary.

Extinction phase analysis: Extinction subject groups (Total extinction, NPA extinction and PA extinction) were analysed separately. SSR analysis was focused on the first 10 seconds of room-location (interval 0-10s), to assess SSR variations due to aversive stimulus anticipation. As for the conditioning phase, SSR amplitude was measured peak-to-peak between the minima and maxima of the studied time interval, for each condition. For each extinction group, two types of analyses were performed in order to assess 1) the mean SSR amplitude

Journal of Pain (ISSN 2996-1793)

obtained for each condition during the whole extinction phase and 2) the SSR amplitude progression throughout the early and late phase of the extinction period.

For the mean SSR amplitude assessment, SSR segments were averaged across all rounds and baseline corrected following the procedure described in the "conditioning phase analysis" paragraph. In addition to the control room, the analyses were performed on the SSR recorded both in rooms where the aversive conditioning was extinguished (NPAOFF and/or PAOFF) and those where it was not (NPAON and/or PAON). A repeated-measures ANOVA was performed with conditions (NPAON, NPAOFF, PAON, PAOFF, CONT, according to each extinction group) as inner-subject factor. Holm-corrected post-hoc t-tests were added when necessary.

For the SSR amplitude progression assessment, in addition to the control room, the SSR analyses focused on extinguished conditions only (NPAOFF and/or PAOFF). To analyze SSR amplitude progression throughout the entire extinction phase, SSR amplitude was measured for every single round. SSR amplitudes of round 1 to round 5 were averaged defining a first period of extinction called EARLY stage; amplitudes of round 6 to round 10 were averaged defining a second period of extinction called LATE. To verify if the observed evolution of SSR between the EARLY and the LATE stage is due to extinction processes rather than habituation effects, the same procedure was performed on the SSR recorded during the conditioning phase. To avoid any confusion, the control room during conditioning phase will be referred as CONTCOND, and that of the extinction phase CONTEXT. A two-way repeated measures ANOVA was performed for each extinction group with conditions (PAOFF, NPAOFF, CONTEXT, CONTCOND) and period (EARLY, LATE) as inner-subject factors. Holm-corrected post-hoc t-tests were added when needed.

Figure 3

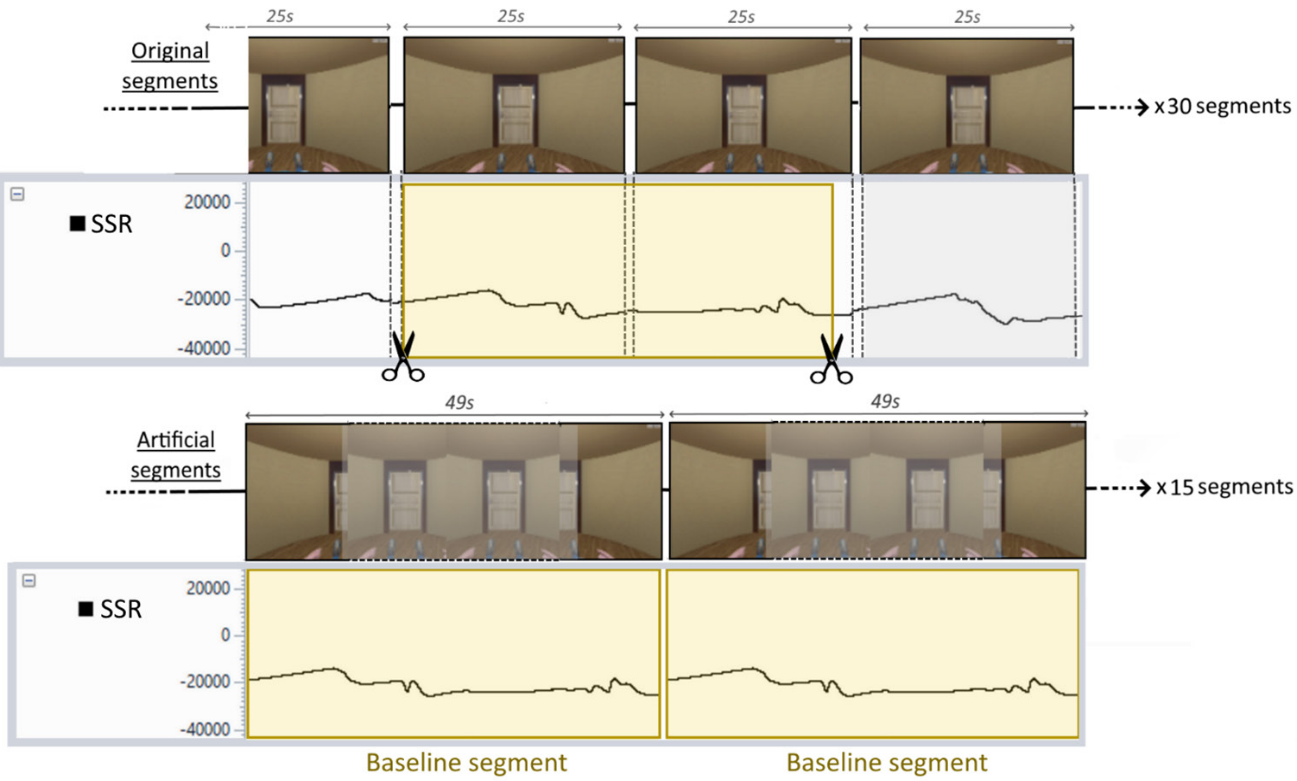

Figure 3 : *Example of Baseline segments creation. Out of the thirty 25-seconds long initial Hallway segments (upper row), a total of fifteen new baseline (BL) segments were created artificially lasting 49-seconds long (lower row) to match condition segments.*

Journal of Pain (ISSN 2996-1793)

Figure 4

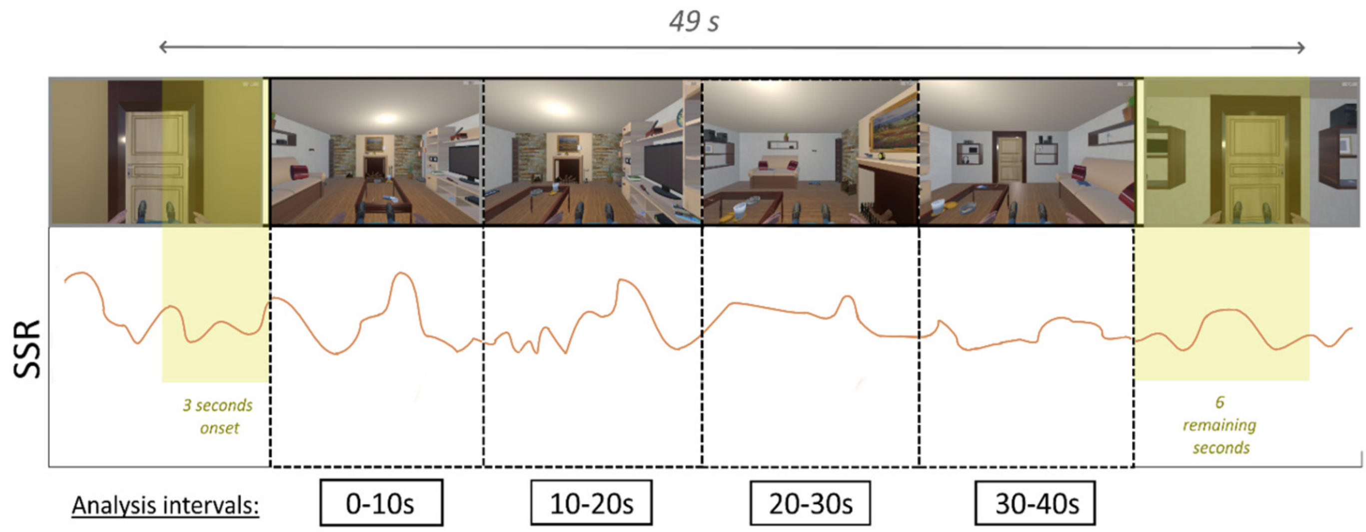

Figure 4 : *Example of averaged and rectified SSR segment division into four equal time- intervals (0-10s, 10-20s, 20-30s, 30-40s). with 3-seconds long onset (to avoid stimulation contamination) and 6 remaining seconds.*

Results

Behavioral results

Immersion quality

Mean score to the location affirmation (4.48 out of 6) and to the possibly affected affirmation (4.31 out of 6)-between "often" and "almost always"- revealed that the subjects' and the avatar's location concurred correctly for 75 % of immersion time and that subjects frequently felt potentially influenced by their surroundings (72% of immersion duration). However, lower mean scores to both the posture and the belonging affirmations (2.63 and 2.03 out of 6 respectively) -between "occasionally" and "half of the time"- revealed that bodily immersion sensations in terms of real-body/avatar merging and complete body ownership were only felt during 39% and 34% of immersion (Figure 5).

Figure 5

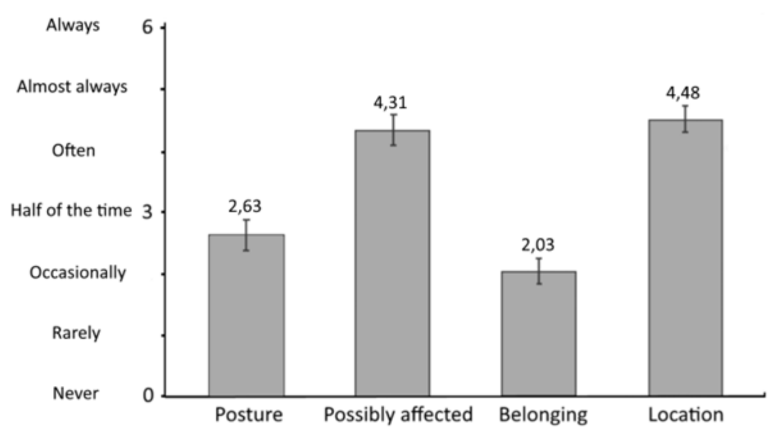

Figure 5 : *Immersion evaluation mean scores for Posture, Possibly affected, Belonging and Location affirmations on a 6 point-scale, 0-points ="Never" and 6-points ="Always".*

Journal of Pain (ISSN 2996-1793)

Stimulation ratings

Results of Student-t test revealed that subject evaluated the painful electro-stimulation (6.3 ±1.8) as more unpleasant than the non-painful aversive sounds (5.7±1.9) (F (1,58)=4.64 p=0.035) (Figure 6). Mean pain intensity rating across subjects was at 5.1±1.6. Note that six subjects out of fifty-nine did not reach the minimum pain threshold (3.0±0.8) during the experiment, due to their consistently low ratings regardless of the stimulation intensity adjustment.

Figure 6

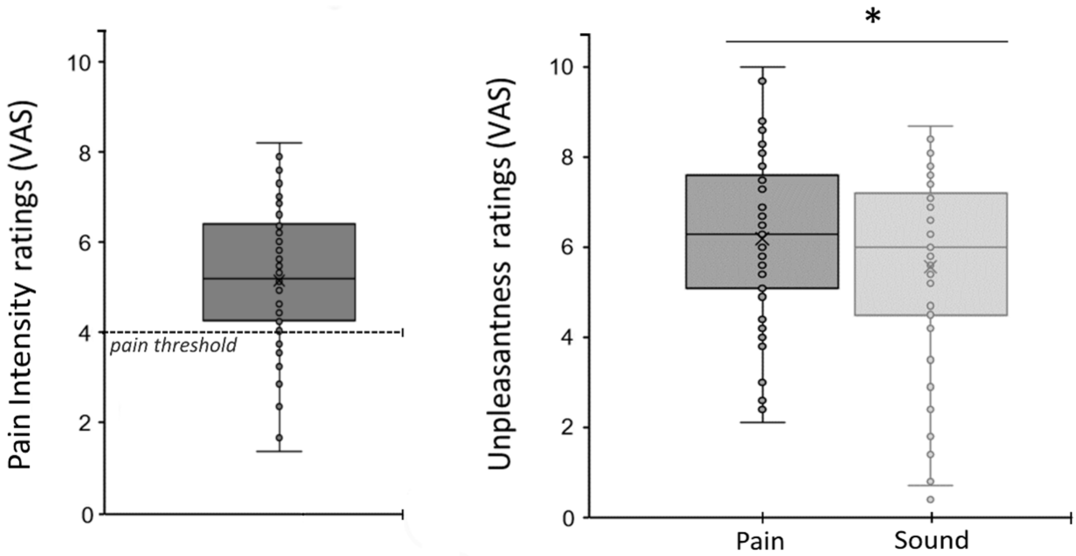

Figure 6 : *Mean intensity and unpleasantness ratings (VAS). On the left: mean intensity ratings across subjects for the painful electro-stimulation; On the right: mean unpleasantness ratings for the painful electro-stimulation (dark grey) and the aversive sound stimulation (light grey) post-conditioning. Individual ratings are represented in dots. *p<0.05*

Room valence

Repeated measures ANOVA revealed both a significant room effect (F(2.58)=5.83; p=0.005), and timepoint effect (F(2.58)=20.94, p<.001) with a significant room*timepoint interaction (F(4.58)=5.09 p=0.001). Results of room*timepoint interaction post-hoc test firstly ensured that subjects assessed equal valence to each room (PA, NPA and CONT) prior conditioning. Post-conditioning, a significant decrease in valence assessment appeared for aversive rooms, that is for the NPA room (t(58)=3.99 p=0.002) and even more so for the PA room (t(58)=4.6 p<.001) compared to the CONT room. No difference was found between PA and NPA rooms. Post-extinction, PA and NPA valence assessment increased approaching CONT room level, hence difference between rooms disappeared (Figure 7A). Detailed posthoc tests are given in table 1.

According to the results presented in Figure 7B, an observational analysis suggests that, during the extinction phase, the aversive room valence is restored to the pre-conditioning level only for the rooms for which the aversive conditioning has been extinguished.

For the Total extinction group, the ANOVA revealed a significant timepoint effect (F(2.19)=2.87, p<.001) and room*timepoint interaction (F(4.19)=4.49, p=0.005). For the two aversive rooms, valence decreased from pre-conditioning to post-conditioning (NPA room: t(19)=4.77, p<.001; PA room: t(19)= 3.88, p=0.006) and restored post-extinction (Figure 7B).

For the NPA extinction group, the ANOVA revealed a significant timepoint effect only (F(2.19)=9.61, p<.001), with an overall significant valence decrease post-conditioning (t(19)=4.15, p<.001) and post-extinction (t(19)=3.29, p=0.004) compared to pre-conditioning valence level (Figure 7B).

For the PA extinction group, the ANOVA revealed a significant timepoint effect (F(2.18)=6.33, p=0.005) and room*timepoint interaction (F(4.18)=7.98, p<0.001). The PA room valence significantly decreased from pre- to post-conditioning (t(18)= 5,29, p<.001), and returned to pre-conditioning level after extinction. For the NPA room, a significant decrease appeared only after extinction compared to pre-conditioning level (t(18)=3.61, p=0.015) (Figure 7B).

Detailed post-hoc tests for each extinction group are given in table 1.

Journal of Pain (ISSN 2996-1793)

Figure 7

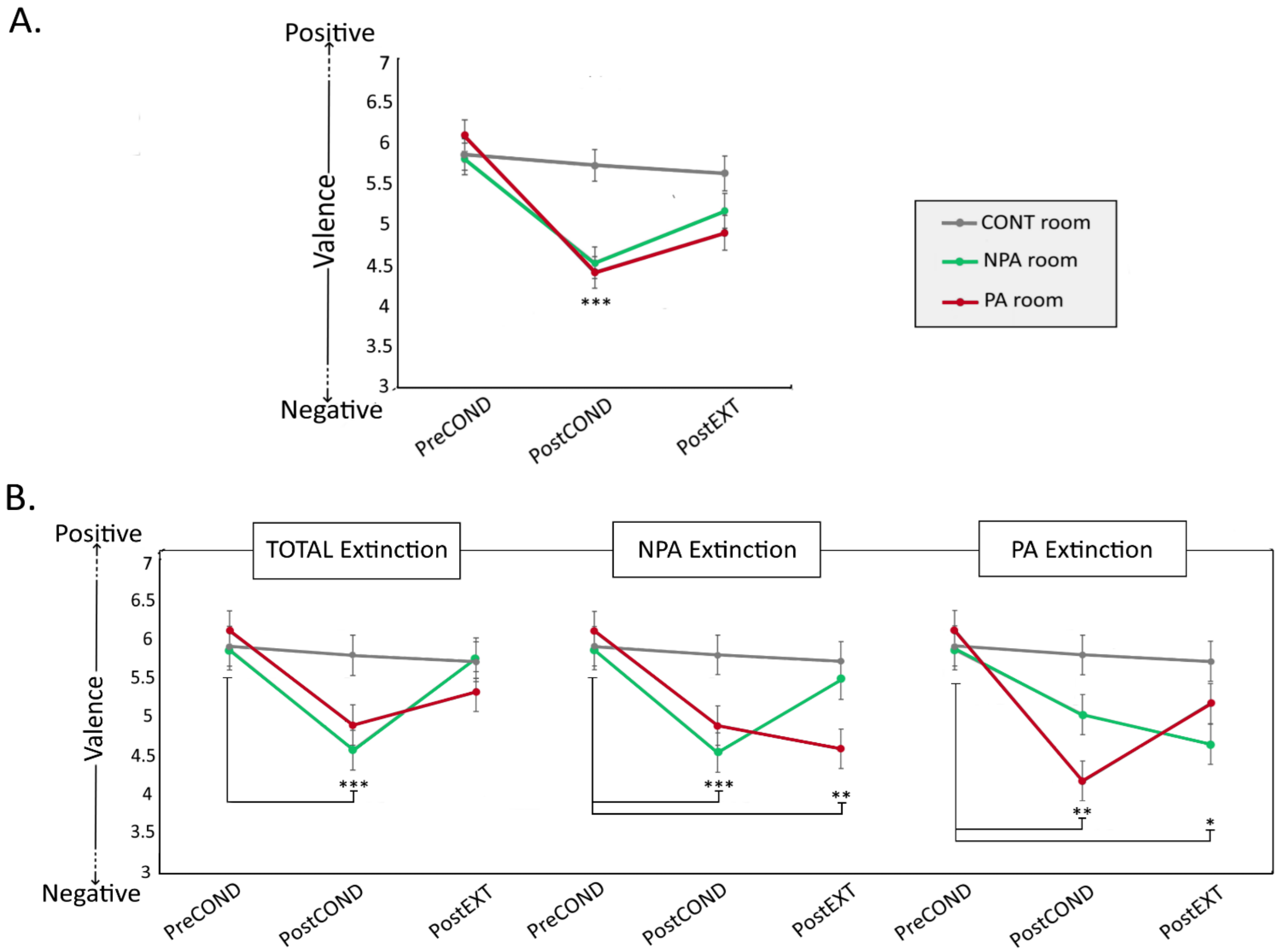

Figure 7 : *Environment perception A. Global valence assessment for each room (PA, NPA and CONT) and each time-point (PreCOND, PostCOND, PostEXT) B. Valence for each extinction group. *p<0.05**p<0.01***p<0.001.*

Journal of Pain (ISSN 2996-1793)

Table 1

			All Subjects (n = 59)		Total Extinction group (n=20)		PA Extinction group (n = 19)		NPA Extinction group (n=20)	
			t(58)	P value	t(19)	P value	t(18)	P value	t(19)	P value
Room	CONT vs NPA		2,78	0,013						
	CONT vs PA		3,11	0,007						
	NPA vs PA		0,33	0,74						
TimePoint	PreCOND vs PostCOND		6,31	<,001	4,29	<,001	3,28	0,007	4,15	<,001
	PreCOND vs PostExt		4,39	<,001	2,26	0,059	2,83	0,015	3,29	0,004
	PostCOND vs PostExt		-1,93	0,057	0,15	0,059	-0,44	0,66	-0,86	0,39
Room*	PreCOND	CONT vs NPA	-0,063	1	-1,44	1,0	-1,14	1		
		CONT vs PA	-0,63	1	1,12	1	-1,14	1		
		NPA vs PA	-0,57	1	0,33	1	-0,001	1		
	PostCOND	CONT vs NPA	3,98	0,002	3,35	0,018	2,19	0,72		
		CONT vs PA	4,55	<,001	2,99	0,095	4,68	<,001		
		NPA vs PA	0,57	1	0,55	1	2,5	0,39		
	PostExt	CONT vs NPA	1,96	0,79	1,99	0,93	2,39	0,47		
		CONT vs PA	2,65	0,17	2,21	0,66	1,24	1		
		NPA vs PA	0,7	1	0,22	1	-1,14	1		
	CONT	PreCOND vs PostCOND	0,42	1	0,22	1	-1,44	1		
		PreCOND vs PostExt	0,7	1	-0,77	1	-0,48	1		
		PostCOND vs PreExt	0,28	0,53	-0,55	1	0,96	1		
	NPA	PreCOND vs PostCOND	4,93	<,001	4,77	<,001	2,41	0,46		
		PreCOND vs PostExt	2,95	0,073	2,66	0,23	3,61	0,015		
		PostCOND vs PreExt	-1,97	0,79	-2,11	0,78	1,2	1		
	PA	PostCOND vs PreExt	6,19	<,001	3,88	0,006	5,29	<,001		
		PreCOND vs PostExt	4,36	<,001	2,55	0,29	2,29	0,58		
		PostCOND vs PreExt	-1,83	0,95	-1,33	1	-3,01	0,094		

Table 1: Detailed results of post-hoc tests of valence assessment (Holm corrected): between rooms (CONT, NPA, PA), timepoints (PreCOND, PostCOND, PostEXT) and room*timepoint interaction for (i) All subjects (n=59), (ii) Total extinction group (n=20), (iii) PA extinction group (n=19) and (iv) NPA extinction group (n=20). Significant p values are written in bold.

Physiological results

Conditioning phase

Example of one subject's mean SSR for each condition as a function of time is given in Figure 8A.

Repeated measures ANOVA revealed that SSR amplitude depended on the condition associated room (F(4.58)=42.8 p<.001) with SSR amplitude of the PAON condition appearing considerably greater compared to all the other situations (CONT: t(58)=11.6 p<.001; PAOFF t(58)=8.71 p<.001; NPAON: t(58)=9.71 p<.001; NPAOFF: t(58)=10.4 p<.001); as well as a greater SSR amplitude in the PAOFF condition compared to CONT (t=2.84 p=0.03) (Figure 8B). Time-interval was also a contributing factor (F=10.9 p<.001) as SSR amplitude during the first ten seconds of room circuits (0-10s) appeared significantly different than SSR amplitude during the remaining 30 seconds (10-20s: t=3.78 p<.001; 20-30s: t=4.88 p<.001; 30-40s: t=4.99 p<.001) with a significant room/time-interval interaction (F=6.63 p<.001). Indeed, SSR amplitude during the PAOFF condition was significantly

Journal of Pain (ISSN 2996-1793)

larger compared to control during the first ten seconds of room circuit only (t=4.09 p=0.007) (Figure 8C), with a slight tendency remaining in the second time-interval (10-20s) (t= 3.46 p=0.075) before decreasing to control level at 20s past room entry. SSR amplitude during the PAON condition was overall significantly larger compared to all other situations (PAOFF, NPAON, NPAOFF, CONT) in all time-intervals (010s, 10-20s, 20-30s and 30-40s) (see table 2).

Note: SSR amplitude of the PA room overshadows what happens in the NPA room. Isolating NPA ON, NPA OFF and CONT, similar results were exposed with an overall room effect (F(2.58)= 6.61 p=0.004), post-hoc t-tests revealing a significant difference between NPA OFF and CONT (t=2.30 p=0.047) and even more so between NPA ON and CONT (t(58) = 3.58 p=0.002)

Figure 8

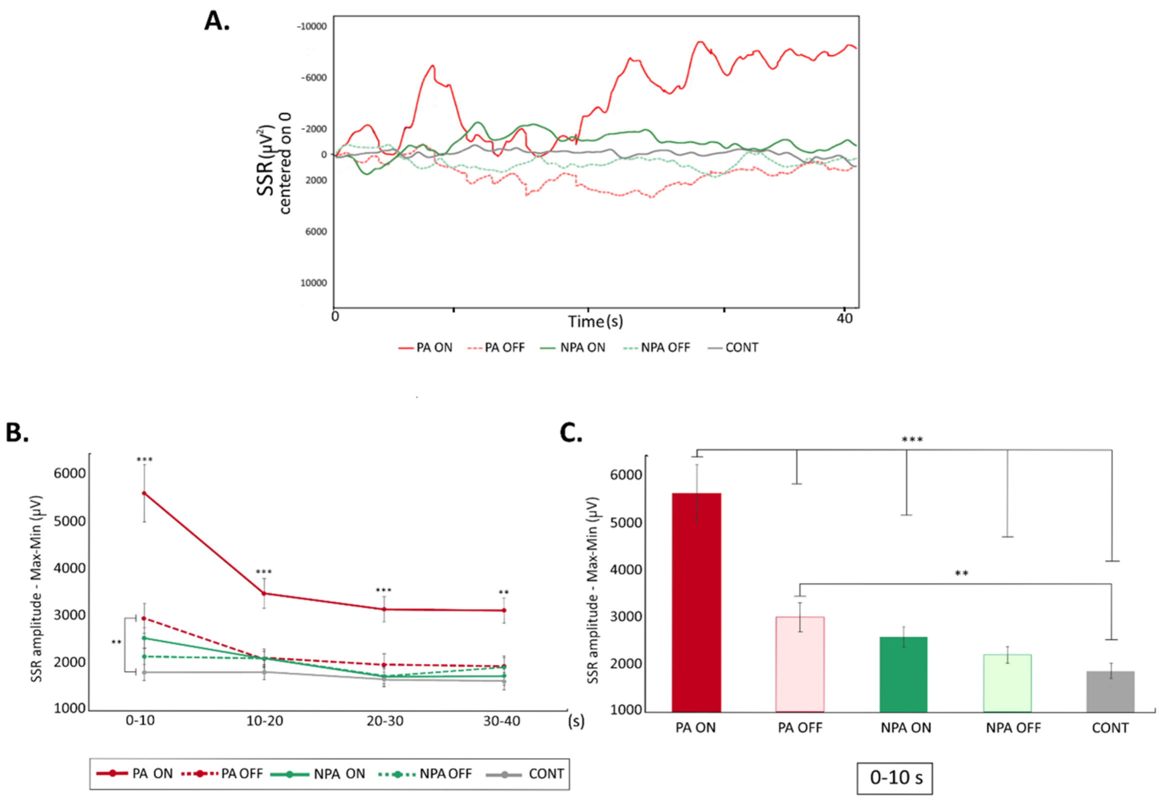

*Figure 8 : A. Example of one subject's mean SSR across conditions (PAON, PAOFF, NPAON, NPAOFF, CONT) throughout the entire duration of a room circuit (49 seconds long). Mean SSRs are baseline corrected and centered at 0 for visualization facilitation B. Mean SSR peak-to- peak amplitude (Max-Min) (µV) for each condition at each time-interval (0-10s, 10-20s, 20- 30s, 30-40s) and C. Mean SSR peak-to-peak amplitude (Max-Min) (µV) during the first 10 seconds of room-circuit (0-10s interval) for each condition **p<0.01 ***p<0.001.*

Table 2

		PA ON	PA OFF		NPA ON		NPA OFF		CONT	
			t(58)	p Value	t(58)	p Value	t(58)	p Value	t(58)	p Value
[00-10s]	PA ON		9,31	<,001	1,04	<,001	12,2	<,001	13,4	<,001
	PA OFF				1,13	1,0	2,86	0,5	4,09	0,007
[10-20s]	PA ON		4,77	<,001	4,53	0,001	4,99	<,001	5,64	<,001
	PA OFF				0,24	1,0	2,22	1,0	3,46	0,075
[20-30s]	PA ON		4,04	0,009	4,84	<,001	5,03	<,001	5,21	<,001
	PA OFF				0,87	1,0	0,99	1,0	1,18	1,0
[30-40s]	PA ON		3,83	0,019	4,62	<,001	3,93	0,013	4,86	<,001
	PA OFF				0,82	1,0	0,099	1,0	1,02	1,0

***Table 2 :** Detailed results of Post-hoc tests (Holm corrected) for SSR peak-to-peak amplitude of the PAON and PAOFF conditions compared to all other situations at all time- intervals*

Journal of Pain (ISSN 2996-1793)

Extinction phase

Mean SSR amplitude: For the total extinction group, analysis of mean SSR amplitude during the first ten seconds of a room circuit revealed no difference between extinguished conditions (PAOFF, NPAOFF) and control (CONT). For the NPA extinction group, repeated measures ANOVA results revealed a room effect (F(3,19)=11.6, p<.001) with mean SSR amplitude being significantly higher for both PAON and PAOFF compared to CONT (t(19)=4.70 p<.001; t(19)=3.72 p=0.002) and the extinguished condition NPAOFF (t(19)=4.49 p<.001; t(19)=3.52 p=0.003). No difference was revealed between NPAOFF and CONT. For the PA extinction group, no difference in mean SSR amplitude was found between conditions, including the non-extinguished condition (NPAON, NPAOFF), the extinguished condition (PAOFF), and control (CONT) (Figure 9A).

SSR amplitude progression assessment: For all three extinction groups, results of repeated measures ANOVAs indicated a period effect (total extinction: F(1,19)=7.94 p=0.013; NPA extinction: F(1,19)=16.1 p=0.001 ; PA extinction: F(1.19)=8,88 p=0.008) with overall SSR amplitudes being significantly larger at the EARLY period of extinction (from round 1 to 5) compared to the LATE period (round 5 to 10) (Figure 9B). However, no statistical difference was found between conditions of the extinction phase (PAOFF, NPAOFF, CONTEXT according to each group) and that of the conditioning phase (CONTCOND), suggesting that the observed SSR decline reflects progressive habituation rather than an extinction effect.

Figure 9

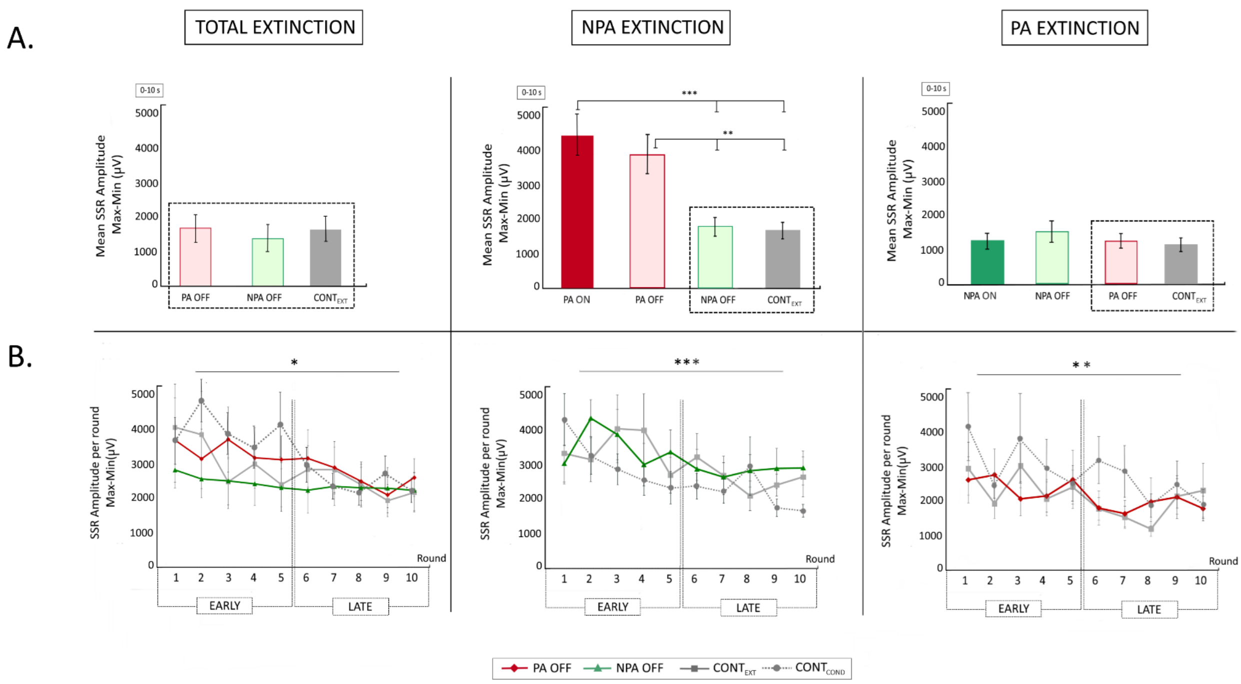

*Figure 9 : SSR peak-to-peak amplitude (µV) during extinction A. Mean SSR amplitude during the 0-10s time interval for each condition of all three extinction groups: total extinction, NPA extinction and PA extinction .Conditions used for further SSR amplitude progression analysis are framed in dotted line, including extinguished conditions (NPAOFF and/or PAOFF) and control (CONT) B. SSR amplitude for every round (round 1 to 5 : EARLY period; round 6 to 10 : LATE period) of the total extinction the NPA extinction and the PA extinction groups*p<0.05**p<0.01***p<0.001*

DISCUSSION

The goal of this study was to build a contextual aversive (painful and non-painful) conditioning protocol by means of an "ecological" environment modelled in immersive Virtual Reality, and demonstrate its usefulness in modulating both psychophysical and physiological measures in a sample lf healthy volunteers. The environment was designed as a three-room apartment (kitchen, living-room, bathroom) allowing to associate each one to a specific stimulus: painful, an electrical tonic stimulation induced on the left-hand; non-painful, a set of aversive noises; or no stimulation, serving as control. Fifty-nine healthy volunteers underwent the conditioning protocol: subjects "entered" 10 times each room in random order, during which the different stimuli were induced in their attributed context. The conditioning phase was followed by an extinction protocol for which three groups of subjects were defined: (i) a total extinction group – both aversive conditionings were extinguished, (ii) a PA extinction group – only the painful aversive conditioning was extinguished and (iii) an NPA extinction group – only the non-painful aversive

Journal of Pain (ISSN 2996-1793)

conditioning was extinguished. Behavioral data (immersion quality, stimuli intensity/unpleasantness and room valence) and electrophysiological data (sympathetic skin response) were recorded in order to validate the usefulness of the iVR protocol in modulating subjective and objective pain-related parameters.

Context perception

Results confirmed a successful contextual post-conditioning modulation of perception, indicated by a strong decline in valence assessment for both aversive conditioned rooms (PA and NPA), compared to the control room (CONT). Recovery of aversive rooms' valence back to near control levels was observed exclusively in the case of extinction. That is, subjects belonging to partial extinction groups (NPA and PA extinction) restored valence of the extinguished room only, thus underlining the effectiveness of extinction on context perception. Previous iVR studies showed the impact of threat conditioning on context valence decay (Kroes et al. 2017, Troger et al. 2012, Glotzbach et al. 2012, Andreatta et al. 2015, 2020), with similar outcomes to our results, however very few have directly compared different types of contextual aversive conditioning, as we did by contrasting painful vs. non-painful aversive input. In a recent cue-conditioning study, researchers investigated behavioral correspondence of negative expectations comparing different threat across sensory modalities - including visceral pain versus aversive sounds as unconditioned stimuli (US) (Koenen et al. 2021). They demonstrated that conditioned negative valence attributed to the conditioned cue stimuli (CS) was consistently correlated with USs emotional valence (unpleasantness rating) for which the CS's paired with visceral pain was enhanced compared to that with aversive sounds. In keeping with subjects' higher pain unpleasantness ratings compared to aversive sounds, associated contexts valence evaluation seems to reflect the degree of aversiveness attributed to each stimulation. Indeed, pain represents an emotionally salient-threat- or stressor (see review Timmers et al. 2019), and in particular pain has the capacity of generating single incident learning, for which memory formation can last a life-time (Apkarian et al. 2009). Accordingly, Schafe et al. highlighted the effectiveness of pain on associative learning and memory in a classical Pavlovian paradigm, as they noted that the more painful the US, the less trials were needed to establish a long-term aversive negative emotion towards the initially neutral CS (Shafe et al. 2001). Although direct comparison of aversive conditionings had no outcome, our results suggest that the pain conditioning specifically seemed to elicit stronger perception modulation of the related context.

Physiological response

Evidence of an effective conditioning was also revealed on physiological levels. Autonomic responses assessed with SSR amplitude were significantly larger for the PA room when the painful stimulation was delivered (PAON) compared to all other conditions (CONT, NPAON, NPAOFF). A greater amplitude was also revealed in the PA room in absence of the painful stimulus (PAOFF) compared to control (CONT), thus manifesting signs of increased pain-related-stress and pain-contextual-related anticipation (Andreatta et al. 2015a, 2017, Neueder et al. 2019, Genheimer et al. 2017, Baas et al. 2004). The increase of SSR amplitude in the PAOFF condition primarily occurred in the first 10 seconds of entering a specific room, only to disappear for the remaining 30 seconds, presumably coordinated with subjects' awareness of stimulus absence. Skin conductance responses (SCR), such as variability and SCR levels, have been positively correlated to experimental and clinical pain (Salameh et al 2022, Sugiminie et al. 2020, Syrjala et al. 2019, Günther et al. 2016, 2013) as well as emotional stress (Günther et al. 2013). Studies have also shown that SCR to painful stimulations were significantly stronger compared to those evoked by non-painful sympathetic stimuli, negatively connotated sound or even emotionally induced stress (Sugiminie et al. 2020, Günther et al. 2016). Larger SSR amplitude during the early period of PAOFF compared to CONT indicates that the pain associated room itself may have induced higher emotional stress. Pain-specificanticipation response when confronting the conditioned environment is proof to an effective contextual pain conditioning. A similar outcome was observed for SSR amplitude of the nonpainful aversive conditioning (NPAON, NPAOFF), however the pain related effect statistically overshadowed this result.

For each extinction group, observational analysis of SSR during all ten rounds revealed a progressive decline in amplitude response for analyzed conditions of the extinction phase (PAOFF and/or NPAOFF, CONTEXT) and of the conditioning phase (CONTCOND). This result was confirmed with a significantly lower mean SSR amplitude during the LATE stage compared to the EARLY stage, all conditions confounded. A decrease of behavioral response when a novel stimulus is presented repeatedly is known as the habituation effect, which involves progressive fading of autonomic responses in anticipation to that stimulation until novelty dissolves (Cohen, 2011). Interestingly, this adaptive behavior can be used as a comparable method to common extinction treatment, where subjects are repeatedly exposed to aversive USs until reduction of customary physiological response, called habituation treatment (Haesen and Vervliet, 2015). Regardless, in our case the lack of discrimination found between conditions across phases suggests that SSR amplitude decline was due to overall habituation to the virtual contexts' stimuli rather than any targeted extinction outcome. Although results of mean

SSR amplitude effectively reflected conditioning processes during acquisition, extinction mechanisms were to a greater extent evidenced in behavioral results.

Immersion quality

Results of immersion evaluation showed that surface level immersion parameters were mostly respected, such as synchronized location with the avatar and sense of possible affection by the environment. However, subjects did not attain complete sense of body ownership towards the avatar including posture merging and belonging sensation, possibly reflecting a weaker sense of immersion.

Among essential parameters of immersive Virtual reality, 3D visual settings represent the basis of any iVR environment, and immersion quality has been initially reported to depend on visual stimuli realism (Hendrix & Barfield 1996, Slater et al. 1995). Studies have shown that enhancing graphic detailing (Hvass et al. 2017, Hendrix & Barfield 1996) and visual dynamic parameters- such as ray-tracing, shadow casting and reflection effect- (Khanna et al. 2006, Slater et al. 2009, Mania and Robinson 2004) result in both improved self-reported sense of presence and stronger physiological fear responses (Hvass et al. 2017, Slater et al. 2009).

However, as iVR technology evolves into a more interactive platform, where users embody active avatars, the likelihood that a stronger immersion quality would rely only on visual rendering is arguable (Steed et al. 2018). Steed et al. describe immersion as a combination of four possible illusions, each classified as degrees of immersion (Steed et al. 2018): place illusion, plausibility illusion, body ownership and agency. Place illusion and plausibility illusion constitute the mere sense of presence and translate respectively the perceptual impression of being in the virtual environment and the illusion that what occurs in the later is real (Slater 2009). In our case, high scores found for location and possibly affected questions would translate the first two degrees of illusion thus assuring a strong sense of presence. Though participants had very little movement range, the correspondence between what was expected (i.e. the explanation and instructions given at the beginning) and what truly occurred was close enough to create the feeling of presence in the apartment. Body ownership and agency however rely on more robust immersion criteria including control of one's virtual body and sense of impact and control over its surrounding environment (Kim et al. 2020). In our case, the lack of body ownership is to be expected due to the lack of stimuli input/output needed to create these impressions. For our experimental settings including multiple measuring and stimulation equipment, these elements could have hardly been added. They are however important to consider for future protocols allowing extended freedom of movement and agency in the virtual apartment. The goal would be to expand immersion and, along these lines, improve ecological quality.

CONCLUSION

The goal of this study was to develop an effective ecological contextual aversive conditioning paradigm in healthy subjects, using immersive Virtual Reality coupled to aversive painful and non-painful stimuli. Aversive conditioning was successfully acquired and demonstrated contextual modulation of both psychophysical (negative perception of conditioned contexts) and physiological responses (sympathetic skin responses to aversive stimulations and to stimulation anticipation when entering associated contexts. The innovative aspect of our protocol was (i) the use of long-lasting stimulation mimicking real-life conditions such as chronic pain, and (ii) using a general context conditioning, rather than a cue-based conditioning based on specific items in the environment. The outcome of this study encourages further use of iVR environments to focus on different research interrogations, still mainly addressed in cue conditioning paradigms, such as explicit memory mechanisms (Dunsmoor and Kroes 2018, Åhs et al. 2015, Connor and Gould 2016), attentional biases (Klein et al. 2021, Oehlberg and Mineka 2011, Koenig et al. 2021), gaze and avoidance behavior (Armstrong et al. 2022, Michalska et al. 2017) and extinction and renewal of threat responses (Andreatta et al. 2020, 2017, Hermann et al. 2016, Milad et al. 2005, Bouton et al. 2004).

Some material is available at:
https://github.com/argitxuCaldichoury/VEProtocol.git

On GitHub:

Word document 1 Contextual conditioning protocol implementation in immersive Virtual Reality. This document explains in detail the material, set up and implementation of the protocol to enable reproduction of experimental settings.

Folder 1 Unity Scripts. This folder contains all scripts coded in Visual Studio and used to implement the experimental flow of the protocol. Specific use of each script is given in the Word document 1.

Video 1 Example of an experimental session illustrating one tour around the virtual apartment. Simulation of a subject's tour circuit around the apartment, presenting all three rooms (Living room, bathroom, kitchen) and the central hallway. An example of a subject's set-up and room/condition association is given at the start of the video. Each condition is color-coded: red for painful aversive (PA), green for non-painful aversive (NPA) and black for control (CONT). The video speed is times two. Note that the video includes the representation of a cognitive task explained in Word document 1.

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Conflicts of interest/Competing interests: The authors declare no competing interests.

Ethics approval: Study approved by the National Ethics Committee (Comité de Protection de Personnes CPP Nord Ouest IV, n° 2019-A01816-51), ClinicalTrials.gov ID: NCT04189965

Consent to participate: Participants of this study were recruited through announcements at the Neuroscience Research Center (CRNL) and universities of Lyon, signed informed consent, and were remunerated for their participation.

Code availability: The code is available at **https://github.com/argitxuCaldichoury/VEProtocol.git**

Data availability statement: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

REFERENCES

1. Åhs, F., Dunsmoor, J.E., Zielinski, D., LaBar, K.S., 2015. Spatial proximity amplifies valence in emotional memory and defensive approach-avoidance. Neuropsychologia 70, 476–485. https://doi.org/10.1016/j.neuropsychologia.2014.12.018

2. Alvarez, R.P., Biggs, A., Chen, G., Pine, D.S., Grillon, C., 2008. Contextual fear conditioning in humans: cortical-hippocampal and amygdala contributions. J Neurosci 28, 6211–6219. https://doi.org/10.1523/JNEUROSCI.1246-08.2008

3. Andersen, S., Thorpe, J., 2009. An IF–THEN theory of personality: Significant others and the relational self. Journal of Research in Personality 43, 163–170. https://doi.org/10.1016/j.jrp.2008.12.040

4. Andreatta, M., Leombruni, E., Glotzbach-Schoon, E., Pauli, P., Mühlberger, A., 2015a. Generalization of Contextual Fear in Humans. Behavior Therapy 46. https://doi.org/10.1016/j.beth.2014.12.008

5. Andreatta, M., Neueder, D., Glotzbach-Schoon, E., Mühlberger, A., Pauli, P., 2017. Effects of context preexposure and delay until anxiety retrieval on generalization of contextual anxiety. Learn. Mem. 24, 43–54. https://doi.org/10.1101/lm.044073.116

6. Andreatta, M., Genheimer, H., Wieser, M.J., Pauli, P., 2020. Context-dependent generalization of conditioned responses to threat and safety signals. Int J Psychophysiol 155, 140–151. https://doi.org/10.1016/j.ijpsycho.2020.06.006

7. Apkarian, A.V., Baliki, M.N., Geha, P.Y., 2009. Towards a theory of chronic pain. Prog Neurobiol 87, 81–97. https://doi.org/10.1016/j.pneurobio.2008.09.018

8. Armstrong, T., Sarawgi, S., Olatunji, B.O., 2012. Attentional bias towards threat in contamination fear: Overt components and behavioral correlates. J Abnorm Psychol 121, 232–237. https://doi.org/10.1037/a0024453

9. Baas, J.M., Nugent, M., Lissek, S., Pine, D.S., Grillon, C., 2004. Fear conditioning in virtual reality contexts: a new tool for the study of anxiety. Biological Psychiatry 55, 1056–1060. https://doi.org/10.1016/j.biopsych.2004.02.024

10. Baddeley, A. D., Emslie, H., & Nimmo-Smith, I. ,1994. The Doors and People Test: A test of visual and verbal recall and recognition. Bury-St-Edmunds, UK: Thames Valley Test Company

11. Baños, R., Botella, C., Garcia-Palacios, A., Martin, H., Perpiñá, C., Alcañiz Raya, M., 2000. Presence and Reality Judgment in Virtual Environments: A Unitary Construct? CyberPsychology & Behavior 3. https://doi.org/10.1089/10949310050078760

12. Botella, C., Serrano, B., Baños, R.M., Garcia-Palacios, A., 2015. Virtual reality exposure-based therapy for the treatment of post-traumatic stress disorder: a review of its efficacy, the adequacy of the treatment protocol, and its acceptability. Neuropsychiatr Dis Treat 11, 2533–2545. https://doi.org/10.2147/NDT.S89542

13. Botella, C., Quero, S., Perpiñá, C., Baños, R., Alcañiz Raya, M., Lozano, J., Rey, A., 1998. Virtual Environments for the Treatment of Claustrophobia. The International journal of Virtual Reality3. https://doi.org/10.20870/IJVR.1998.3.3.2626

14. Botella, C., Fernández-Álvarez, J., Guillén, V., García-Palacios, A., Baños, R., 2017. Recent Progress in Virtual Reality Exposure Therapy for Phobias: A Systematic Review. Curr Psychiatry Rep 19, 42. https://doi.org/10.1007/s11920-017-0788-4

15. Bouton, M., 2004. Bouton ME. Context and behavioral processes in extinction. Learn Mem 11: 485-494.

Learning & memory (Cold Spring Harbor, N.Y.) 11, 485–94. https://doi.org/10.1101/lm.78804

16. Spielberger. Canadian Journal of Behavioural Science / Revue canadienne des sciences du comportement. https://doi.org/10.1037/h0078881

17. Cipresso, P., Giglioli, I.A.C., Raya, M.A., Riva, G., 2018. The Past, Present, and Future of Virtual and Augmented Reality Research: A Network and Cluster Analysis of the Literature. Frontiers in Psychology 9.

18. Connor, D.A., Gould, T.J., 2016. The role of working memory and declarative memory in trace conditioning. Neurobiology of Learning and Memory 134, 193–209. https://doi.org/10.1016/j.nlm.2016.07.009

19. Curzon, P., Rustay, N.R., Browman, K.E., 2009. Cued and Contextual Fear Conditioning for Rodents, in: Buccafusco, J.J. (Ed.), Methods of Behavior Analysis in Neuroscience, Frontiers in Neuroscience. CRC Press/ Taylor & Francis, Boca Raton (FL).

20. Cruz-Neira, C., Sandin, D., DeFanti, T., 1993. Surround-Screen Projection-Based Virtual Reality: The Design and Implementation of the CAVE. Presented at the ACM SIGGRAPH, pp. 135–142. https://doi.org/10.1145/166117.166134

21. Dunsmoor, J.E., Murphy, G.L., 2015. Categories, concepts, and conditioning: how humans generalize fear. Trends Cogn Sci 19, 73–77. https://doi.org/10.1016/j.tics.2014.12.003

22. Dunsmoor, J.E., Kroes, M.C.W., 2019. Episodic memory and Pavlovian conditioning: ships passing in the night. Curr Opin Behav Sci 26, 32–39. https://doi.org/10.1016/j.cobeha.2018.09.019

23. Faasse, K., Johnson, M., 2008. The role of associative learning and pain. Ngau Mamae Quarterly Publication of the New Zealand Pain Society 8, 12–19.

24. Fonteyne, R., Vervliet, B., Hermans, D., Baeyens, F., Vansteenwegen, D., 2010. Exposure to the context and removing the unpredictability of the US: Two methods to reduce contextual anxiety compared. Biological Psychology 85, 361–369. https://doi.org/10.1016/j.biopsycho.2010.08.007

25. Genheimer, H., Andreatta, M., Asan, E., Pauli, P., 2017. Reinstatement of contextual conditioned anxiety in virtual reality and the effects of transcutaneous vagus nerve stimulation in humans. Sci Rep 7, 17886. https://doi.org/10.1038/s41598-017-18183-3

26. Günther, A.C., Schandl, A.R., Berhardsson, J., Bjärtå, A., Wållgren, M., Sundin, Ö., Alvarsson, J., Bottai, M., Martling, C.-R., Sackey, P.V., 2016. Pain rather than induced emotions and ICU sound

27. increases skin conductance variability in healthy volunteers. Acta Anaesthesiol Scand 60, 1111–1120. https://doi.org/10.1111/aas.12751

28. Günther, A.C., Bottai, M., Schandl, A.R., Storm, H., Rossi, P., Sackey, P.V., 2013. Palmar skin conductance variability and the relation to stimulation, pain and the motor activity assessment scale in intensive care unit patients. Critical Care 17, R51. https://doi.org/10.1186/cc12571

29. Harvie, D.S., Moseley, G.L., Hillier, S.L., Meulders, A., 2017. Classical Conditioning Differences Associated With Chronic Pain: A Systematic Review. J Pain 18, 889–898. https://doi.org/10.1016/j.jpain.2017.02.430/

30. Hermann, A., Stark, R., Milad, M.R., Merz, C.J., 2016. Renewal of conditioned fear in a novel context is associated with hippocampal activation and connectivity. Soc Cogn Affect Neurosci 11, 1411–1421. https://doi.org/10.1093/scan/nsw047

31. Houtekamer, M.C., Henckens, M.J.A.G., Mackey, W.E., Dunsmoor, J.E., Homberg, J.R., Kroes, M.C.W., 2020. Investigating the efficacy of the reminder-extinction procedure to disrupt contextual threat memories in humans using immersive Virtual Reality. Sci Rep 10, 16991. https://doi.org/10.1038/s41598-020-73139-4

32. Huff, N.C., Zeilinski, D.J., Fecteau, M.E., Brady, R., LaBar, K.S., 2010. Human fear conditioning conducted in full immersion 3-dimensional virtual reality. J Vis Exp 1993. https://doi.org/10.3791/1993

33. Hvass, J., Larsen, O., Vendelbo, K., Nilsson, N., Nordahl, R., Serafin, S., 2017. Visual realism and presence in a virtual reality game, in: 2017 3DTV Conference: The True Vision - Capture, Transmission and Display of 3D Video (3DTV-CON). pp. 1–4. https://doi.org/10.1109/3DTV.2017.8280421

34. Johnson, L.M., Zautra, A.J., Davis, M.C., 2006. The role of illness uncertainty on coping with fibromyalgia symptoms. Health Psychology 25, 696–703. https://doi.

org/10.1037/0278-6133.25.6.696

35. Keefe, F.J., Huling, D.A., Coggins, M.J., Keefe, D.F., Rosenthal, M.Z., Herr, N.R., Hoffman, H.G., 2012. Virtual Reality for Persistent Pain: A New Direction for Behavioral Pain Management. Pain 153, 2163– 2166. https://doi.org/10.1016/j.pain.2012.05.030

36. Kenney, J.W., Scott, I.C., Josselyn, S.A., Frankland, P.W., 2017. Contextual fear conditioning in zebrafish. Learn Mem 24, 516–523. https://doi.org/10.1101/lm.045690.117

37. Khanna, P., Yu, I., Mortensen, J., Slater, M., 2006. Presence in response to dynamic visual realism: A preliminary report of an experiment study. Presented at the Paper Presented at the 13th ACM Symposium Virtual Reality Software and Technology, VRST'06, pp. 364–367. https://doi.org/10.1145/1180495.1180569

38. Klein, Z., Ginat-Frolich, R., Barry, T., Shechner, T., 2021. Effects of increased attention allocation to threat and safety stimuli on fear extinction and its recall. Journal of Behavior Therapy and Experimental Psychiatry 72, 101640. https://doi.org/10.1016/j.jbtep.2021.101640

39. Kim, K., Rosenthal, M.Z., Zielinski, D.J., Brady, R., 2014. Effects of virtual environment platforms on emotional responses. Computer Methods and Programs in Biomedicine 113, 882–893. https://doi.org/10.1016/j.cmpb.2013.12.024

40. Kim, S.-Y., Park, H., Jung, M., Kim, K.K., 2020. Impact of Body Size Match to an Avatar on the Body Ownership Illusion and User's Subjective Experience. Cyberpsychol Behav Soc Netw 23, 234–241. https://doi.org/10.1089/cyber.2019.0136

41. Koenig, S., Körfer, K., Lachnit, H., Glombiewski, J.A., 2021. An attentional perspective on differential fear conditioning in chronic pain: The informational value of safety cues. Behaviour Research and Therapy 144, 103917. https://doi.org/10.1016/j.brat.2021.103917

42. Kroes, M.C.W., Dunsmoor, J.E., Mackey, W.E., McClay, M., Phelps, E.A., 2017. Context conditioning in humans using commercially available immersive Virtual Reality. Sci Rep 7, 8640. https://doi.org/10.1038/s41598-017-08184-7

43. Lombard, M., Ditton, T., 1997. At the Heart of It All: The Concept of Presence. Journal of Computer-Mediated Communication 3, 0–0. https://doi.org/10.1111/j.1083-6101.1997.tb00072.x

44. Madden, V.J., Bellan, V., Russek, L.N., Camfferman, D., Vlaeyen, J.W.S., Moseley, G.L., 2016. Pain by Association? Experimental Modulation of Human Pain Thresholds Using Classical Conditioning. J Pain 17, 1105–1115. https://doi.org/10.1016/j.jpain.2016.06.012

45. Mania, K., Robinson, A., 2004. The effect of quality of rendering on user lighting impressions and presence in virtual environments. pp. 200–205. https://doi.org/10.1145/1044588.1044629

46. Mazza, S., Frot, M., Rey, A.E., 2018. A comprehensive literature review of chronic pain and memory. Prog Neuropsychopharmacol Biol Psychiatry 87, 183–192. https://doi.org/10.1016/j.pnpbp.2017.08.006

47. Meulders, A., Vansteenwegen, D., Vlaeyen, J.W.S., 2011. The acquisition of fear of movement-related pain and associative learning: a novel pain-relevant human fear conditioning paradigm. Pain 152, 2460– 2469. https://doi.org/10.1016/j.pain.2011.05.015

48. Meulders, A., Vlaeyen, J.W.S., 2013. Mere intention to perform painful movements elicits fear of movement-related pain: an experimental study on fear acquisition beyond actual movements. J Pain 14, 412–423. https://doi.org/10.1016/j.jpain.2012.12.014

49. Meulders, A., Vandael, K., Vlaeyen, J.W.S., 2017. Generalization of Pain-Related Fear Based on Conceptual Knowledge. Behavior Therapy 48, 295–310. https://doi.org/10.1016/j.beth.2016.11.014

50. Meulders, A., 2020. Fear in the context of pain: Lessons learned from 100 years of fear conditioning research. Behaviour Research and Therapy 131, 103635. https://doi.org/10.1016/j.brat.2020.103635

51. Meulders, A., Meulders, M., Stouten, I., De Bie, J., Vlaeyen, J.W.S., 2017. Extinction of Fear Generalization: A Comparison Between Fibromyalgia Patients and Healthy Control Participants. J Pain 18, 79–95. https://doi.org/10.1016/j.jpain.2016.10.004

52. Michalska, K.J., Machlin, L., Moroney, E., Lowet, D.S., Hettema, J.M., Roberson-Nay, R., Averbeck, B.B., Brotman, M.A., Nelson, E.E., Leibenluft, E., Pine, D.S., 2017. Anxiety symptoms and children's eye gaze during fear learning. J Child Psychol Psychiatry 58, 1276–1286.

https://doi.org/10.1111/jcpp.12749

53. Milad, M., Orr, S., Roger, P., Rauch, S., 2005. Context modulation of memory for fear extinction in humans. Psychophysiology 42, 456–64. https://doi.org/10.1111/j.1469-8986.2005.00302.x

54. Moseley, G.L., Vlaeyen, J.W.S., 2015. Beyond nociception: the imprecision hypothesis of chronic pain. Pain 156, 35–38. https://doi.org/10.1016/j.pain.0000000000000014

55. Mühlberger, A., Andreatta, M., Ewald, H., Glotzbach-Schoon, E., Tröger, C., Baumann, C., Reif, A., Deckert, J., Pauli, P., 2014. The BDNF Val66Met Polymorphism Modulates the Generalization of Cued Fear Responses to a Novel Context. Neuropsychopharmacol 39, 1187–1195. https://doi.org/10.1038/npp.2013.320

56. Neueder, D., Andreatta, M., Pauli, P., 2019. Contextual Fear Conditioning and Fear Generalization in Individuals With Panic Attacks. Frontiers in Behavioral Neuroscience 13.

57. Oehlberg, K., Mineka, S., 2011. Fear Conditioning and Attention to Threat: An Integrative Approach to Understanding the Etiology of Anxiety Disorders. Associative Learning and Conditioning Theory: Human and Non-human Applications 44–78. https://doi.org/10.1093/acprof:oso/9780199735969.003.0020

58. Peyron R, Garcı´a-Larrea L, Gre´goire MC, Convers P, Lavenne F, Veyre L, et al. Allodynia after lateral-medullary (Wallenberg) infarct. A PET study. Brain 1998; 121: 345–56.

59. Reichenberger, J., Pfaller, M., Mühlberger, A., 2020. Gaze Behavior in Social Fear Conditioning: An Eye-Tracking Study in Virtual Reality. Frontiers in Psychology 11.

60. Riva, G., 2005. Virtual reality in psychotherapy: review. Cyberpsychol Behav 8, 220–230; discussion 231-240. https://doi.org/10.1089/cpb.2005.8.220

61. Salameh C, Perchet C, Hagiwara K, Garcia-Larrea L. Sympathetic skin response as an objective tool to estimate stimulus-associated arousal in a human model of hyperalgesia. Neurophysiol Clin. 2022 Nov;52(6):436-445. doi: 10.1016/j.neucli.2022.10.002

62. Sanchez-Vives, M.V., Slater, M., 2005. From presence to consciousness through virtual reality. Nat Rev Neurosci 6, 332–339. https://doi.org/10.1038/nrn1651

63. Schroyens, N., Sigwald, E.L., Van Den Noortgate, W., Beckers, T., Luyten, L., 2021. Reactivation- Dependent Amnesia for Contextual Fear Memories: Evidence for Publication Bias. eNeuro 8, ENEURO.0108-20.2020. https://doi.org/10.1523/ENEURO.0108-20.2020

64. Sjouwerman, R., Niehaus, J., Lonsdorf, T.B., 2015. Contextual Change After Fear Acquisition Affects Conditioned Responding and the Time Course of Extinction Learning—Implications for Renewal Research. Frontiers in Behavioral Neuroscience 9.

65. Slater, M., 2009. Place illusion and plausibility can lead to realistic behaviour in immersive virtual environments. Philos Trans R Soc Lond B Biol Sci 364, 3549–3557. https://doi.org/10.1098/rstb.2009.0138

66. Slater, M., Khanna, P., Mortensen, J., Yu, I., 2009. Visual Realism Enhances Realistic Response in an Immersive Virtual Environment. IEEE computer graphics and applications 29, 76–84. https://doi.org/10.1109/MCG.2009.55

67. Slater, M., Usoh, M., Steed, A., 1995. Taking steps: The influence of a walking technique on presence in virtual reality. ACM Transactions on Computer-Human Interaction (TOCHI) 2, 201–219. https://doi.org/10.1145/210079.210084

68. Spielberger, C. D., Gorsuch, R. L., Lushene, R., Vagg, P. R., & Jacobs, G. A. (1983). Manual for the State-Trait Anxiety Inventory. Palo Alto, CA: Consulting Psychologists Press.

69. Steed, A., Pan, Y., Watson, Z., Slater, M., 2018. "We Wait"—The Impact of Character Responsiveness and Self Embodiment on Presence and Interest in an Immersive News Experience. Frontiers in Robotics and AI 5.

70. Sundar, S.S., Xu, Q., Bellur, S., 2010. Designing interactivity in media interfaces: A communications perspective. Presented at the Proceedings of the Conference on Human Factors in Computing Systems, pp. 2247–2256. https://doi.org/10.1145/1753326.1753666

71. Sutherland, 1965. The Ultimate Display. Multimedia: From Wagner to Virtual Reality. New York, NY: Norton

72. Sugimine, S., Saito, S., Takazawa, T., 2020. Normalized skin conductance level could differentiate physical pain

stimuli from other sympathetic stimuli. Sci Rep 10, 10950. https://doi.org/10.1038/s41598- 020-67936-0

73. Syrjala, E., Jiang, M., Pahikkala, T., Salantera, S., Liljeberg, P., 2019. Skin Conductance Response to Gradual-Increasing Experimental Pain. Annu Int Conf IEEE Eng Med Biol Soc 2019, 3482–3485. https://doi.org/10.1109/EMBC.2019.8857776

74. Vansteenwegen, D., Iberico, C., Vervliet, B., Marescau, V., Hermans, D., 2008. Contextual fear induced by unpredictability in a human fear conditioning preparation is related to the chronic expectation of a threatening US. Biological Psychology 77, 39–46. https://doi.org/10.1016/j.biopsycho.2007.08.012

75. Vlaeyen, J.W.S., 2015. Learning to predict and control harmful events: chronic pain and conditioning. Pain 156, S86–S93. https://doi.org/10.1097/j.pain.0000000000000107

76. Yu, J., Naoi, T., Sakaguchi, M., 2021. Fear generalization immediately after contextual fear memory consolidation in mice. Biochemical and Biophysical Research Communications 558, 102–106. https://doi.org/10.1016/j.bbrc.2021.04.072