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# Validating the Threat of a Virtual Reality Clinical Environment: A Mixed Methods Study

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**Featured Application: Featured Application: We have validated two clinical virtual reality environments, both with varying levels of threat, for use in future health research.**

**Abstract:** There is an increasing need for virtual reality (VR) health applications. In the field of pain, VR has been used mainly as a distraction, with minimal use of VR to answer basic clinical questions. Pain is multifactorial and inherently threatening. Our lab recently designed two VR clinical environments with varying threat values; the present study sought to validate these environments. Subjects were randomly allocated into either the threatening or non-threatening VR consultation room and both subjective (threat questionnaire) and physiological (salivary cortisol) measurements were taken. As hypothesised, subjects in the threat condition recorded a higher threat score ($p < 0.001$; effect size = 0.76). There was a cortisol change across time in the threat condition ($\chi^2(2) = 13.83$, $p < 0.001$), but there were unexpected decreases at both 20 ($p = 0.001$) and 26 min ($p = 0.03$) following VR. While the physiological findings need further clarification, this study provides some validation of the threat value of our VR clinical tools. As such, these VR environments can potentially be used in pain experiments to help better our understanding of basic pain mechanisms. It is only with such understanding that we might offer new avenues for pain management.

**Keywords:** virtual reality; threat; pain; clinical environment; cortisol; mixed methods

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**Citation:** O'Neill, D.; Titmus, M.; Lamont, W.; Teh, W.H.; Perimal, E.; Di Pietro, F. Validating the Threat of a Virtual Reality Clinical Environment: A Mixed Methods Study. *Appl. Sci.* **2024**, *14*, 10009. https://doi.org/10.3390/app142110009

Academic Editor: Andrea Prati

Received: 19 August 2024
Revised: 18 October 2024
Accepted: 28 October 2024
Published: 2 November 2024

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## 1. Introduction

Virtual reality (VR) can produce high-resolution and cost-effective simulations for health applications. There is an increasing need for VR in medicine as it has the potential to provide immersive environments in which to answer clinical questions. In the field of pain, VR has been used to distract patients from pain, such as during paediatric vaccinations [1] or dental interventions [2]; for a recent review, see [3]. It has also been determined that immersive VR can increase pain tolerance limits [4]. Less explored than these clinical questions, however, are the fundamental questions that VR might be able to help answer. Can VR contribute to our understanding of the mechanisms underpinning pain? It is well recognised that pain is multifactorial, with biological, social and psychological mechanisms all contributing to the pain experience [5,6]. Pain has been proposed as an inherently threatening experience, with threat to the self posited as being crucial for pain to be experienced by an individual [7,8]. Humans appear to be sensitive to threat cues in their environment. For example, there is evidence that a red light (associated with threat) results in more intense pain from an experimental noxious stimulus, as compared to a blue light [9].

Participants have also reported higher pain ratings in response to experimental heat pain in a magnetic resonance imaging scanner environment than in a laboratory, indicating that the clinical environment was sufficiently threatening to alter pain perception [10]. However, modelling threat in a clinical setting is difficult, and the role of threat in pain is challenging to test empirically.

VR presents an opportunity for authentic and immersive clinical environments for experiments that might aim to elucidate the important contributors to pain. We recently developed two VR environments, designed to mimic clinical consultation rooms with varying levels of threat. The VR clinical environments hold great potential to be used in experimental pain research, but they first need to be validated, and their intended and hypothesised threat value must be confirmed. One of the most commonly measured physiological parameters in studies on the effect of one's environment is salivary cortisol, a marker of stress or threat [11]. The aim of this study was to validate the threat value of two newly created VR clinical environments using a mixed methods approach: subjectively, via a questionnaire, and physiologically, via the concentration of salivary cortisol.

## 2. Materials and Methods

### 2.1. Subjects

A sample of healthy subjects aged between 18 and 45 years was recruited via social media and campus-wide flyers at Curtin University, Western Australia. Subjects were excluded if they reported previous discomfort with VR use, for example, motion sickness or vertigo. Further exclusion criteria included current or past conditions or activity that may interfere with cortisol secretion, e.g., epilepsy, mood or substance use disorder, chronic fatigue, hormonal disorder (e.g., Cushing syndrome or adrenal insufficiency), psychiatric or neurological disorder, any use of psychotropic or medications that may affect the stress response (e.g., corticosteroids), heart disease or hypertension, smoking, pregnancy or breast-feeding, or alcohol intake greater than 30 units per week. Subjects gave written, informed consent and were advised that they were free to withdraw from the study at any point without prejudice. Ethics approval was granted by the Human Research Ethics Committee at Curtin University (HREC 2023-0226). All procedures conformed to the Declaration of Helsinki.

### 2.2. Sample Size

We aimed to recruit a convenience sample of 15 subjects in each group. A sample size calculation was performed a priori in G power (version 3.1.9.4) [12]. The calculation was based on detecting a moderate change in the salivary cortisol concentration (power = 0.80; $\alpha = 0.05$), as per the work of Martens et al., who evaluated salivary cortisol concentrations following exposure to a stressful virtual reality environment [13]; they reported a large effect with groups of 13 and 15.

### 2.3. Study Design

In a between-group design, subjects attended one testing session of up to 90 min in duration. Testing sessions were scheduled 5 h after the subjects' normal waking time to control for diurnal variations in salivary cortisol [14,15]. Upon arrival, subjects were randomly allocated into either the threatening ('threat') or non-threatening ('non-threat') VR clinical consultation room. Subjects were blinded as to their allocation.

The overall protocol is outlined in Figure 1. Testing was conducted in a $4 \times 4$ m space within a Clinic room in the Health and Wellness Centre at Curtin University. Firstly, subjects were given a ten-minute relaxation period, intended to mitigate any stress associated with research participation (e.g., driving, parking, or finding the research venue). An interview was conducted to collect demographic information and confirm subject eligibility. Prior to being exposed to the allocated VR experimental condition, all subjects were first exposed to an 'introduction' room, plain and devoid of features, to familiarise the subjects with VR and the completion of a virtual questionnaire. A custom questionnaire was given to

subjects while in the introduction room (1QT; as training only), and the same questionnaire (2QT) was given to subjects while they were immersed in the respective VR experimental conditions to assess the subjects' perception of threat associated with each VR environment. Three salivary cortisol collections were taken as physiological measurements of threat: at baseline (before VR exposure; cortisol0) and at both 20 min (cortisol20) and 26 min (cortisol26) after the commencement of exposure to the VR experimental condition.

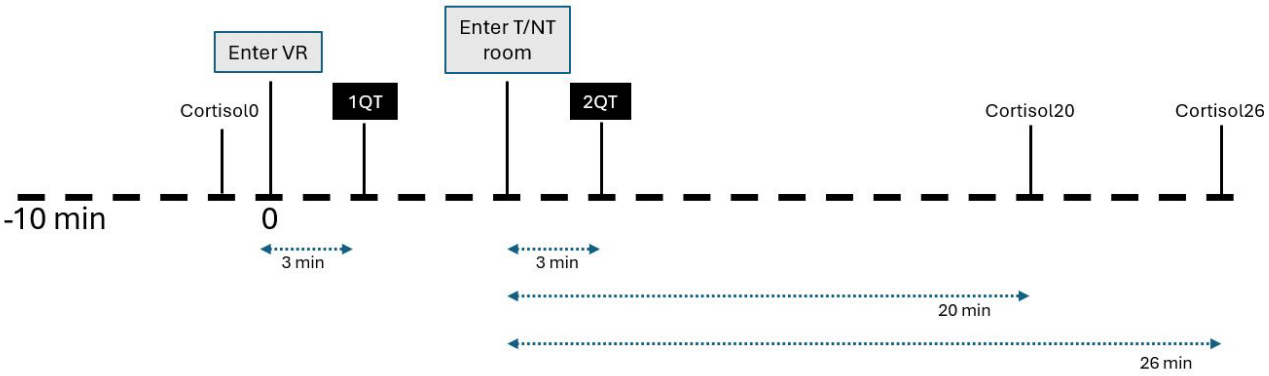



**Figure 1.** An outline of the study protocol. Cortisol0 denotes the cortisol measurement pre-virtual reality (VR) exposure; cortisol20, 20 min post-VR exposure; cortisol26, 26 min post-VR exposure. T, threatening condition; NT, non-threatening condition. 1QT (training only) and 2QT, administration of the same subjective threat questionnaire. Note, subjects exited the VR room after the completion of 1QT/2QT.

### *2.4. VR Environments*

Subjects were placed into the VR environments via an untethered head-mounted display (Meta Quest 2, San Francisco, CA, USA) running Windows operating software (Version 11, Microsoft Corporation, Washington, DC, USA). The VR environment was rendered by a gaming laptop (ASUS TUf A15; 16GB memory; NVIDIA GeForce RTX 3070 graphics card; AMD Ryzen 7 processor, Taipei, Taiwan) using the Unity application (Unity Applications, San Francisco, CA, USA). Data were transmitted to the head-mounted display by a wireless router (ASUS RT-AX53U, Taipei, Taiwan). A stereo image was presented on the headset screen with a 93° vertical field of view and 97° horizontal field of view, with a resolution of 1832 × 1920, allowing subjects to survey the environment with 6 degrees of freedom. The image was updated at 90Hz. Headset-integrated speakers relayed spatially positioned sounds of the simulations and the automated instructions.

Three VR rooms were developed by the team at Curtin University's Hub for Immersive Visualisation and e-Research (HIVE) using the Unity application (Version 2021.3.14f1). The introduction room had a white colour scheme with simple lighting and no accompanying soundscape and was furnished with a single chair. The two experimental clinical rooms were designed to be threatening or non-threatening by the inclusion of a range of visual and auditory features. In the 'threat' VR clinical room, cool fluorescent and flickering lighting was used in conjunction with an unnatural, harsh colour scheme (i.e., red and orange [16,17]). Subjects were exposed to mechanical noise and external voices, such as the sound of a baby crying, played through the headset [18]. The threat room furnishings included messy paperwork strewn over a desk, an overflowing rubbish bin, dead pot plants and a low-back radiograph on a computer display, which is demonstrated to induce stress [19] (Figure 2a). The 'non-threat' VR clinical room included an open window with a view to the natural environment, which also provided natural diffuse lighting and the soundscape of trees rustling and birds chirping. The room was designed with a biophilic colour scheme and contained plants, a pleasant landscape painting and children's artwork, and softer furnishings [20–26] (Figure 2b).



(a) (b)

**Figure 2.** Still images of the two virtual reality (VR) clinical environments: (**a**) an image of the threatening VR consultation room, with a dishevelled clinical space designed to induce stress in a patient, and (**b**) the non-threatening VR consultation room, with an emphasis on natural elements and a calming and unthreatening design.

The VR simulation began outside the introduction room, and automated instructions asked subjects to enter and familiarise themselves with their virtual environment. Although subjects could walk around the virtual environment and immerse themselves in sights and sounds, the environments were passive, i.e., no interaction occurred. After 3 min, 1QT was administered within the VR environment, so subjects could practice completing the virtual questionnaire. Subjects were given one minute to complete 1QT, after which time they were asked to exit the introduction room. The subject then entered their allocated threat or non-threat VR clinical consultation room, and after 3 min, 2QT was administered using the same interface as for 1QT. Subjects were given one minute to complete 2QT and then were asked to exit the experimental VR room.

### *2.5. Measuring the Threat Response*

1QT and 2QT assessed the feelings that the subjects experienced when immersed in the VR conditions. Six adjectives were presented in the following order: threatening, calming, safe, stressful, unpleasant and reassuring. A 5-point Likert scale of response options was given (1 = Strongly Disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, 5 = Agree). The questionnaire was designed to be simple and quick to administer within the VR setting, using both negative [27–29] and positive adjectives [30,31] chosen from the relevant pain and psychology literature. The questionnaire was designed for the current study and was not previously validated.

#### 2.5.1. Cortisol Sample Collection

Salivary cortisol was self-collected using SalivaBio Collection Aid passive drool tubes (Salimetrics, LLC, Carlsbad, CA, USA). Prior to collection, subjects were shown an instructional video on how to correctly collect a sample and how to distribute it into the pre-labelled collection cryovials. Subjects were asked to produce 1.5 mL of saliva within 5 min; however, a minimum volume of 0.5 mL was accepted. The time and date of specimen collection were recorded, and all samples were stored at −20 °C and thawed prior to analysis.

#### 2.5.2. Cortisol Sample Preparation and Analysis

A commercially available enzyme-linked immunosorbent assay (ELISA) kit (Salimetrics, LLC, Carlsbad, CA, USA) was used to quantitate salivary cortisol, based on the protocol recommended by the manufacturer. ELISA is a commonly used and validated biochemical assay that quantitates the presence of a target product (e.g., cortisol) in a solu-

tion. Samples were analysed in duplicate, with a new standard curve completed for each plate. Briefly, 25 µL volumes of the standards, controls and saliva samples were added into the appropriate wells of a 96-well microtiter plate. Then, 200 µL of the diluted conjugate solution (1:1600 diluted enzyme conjugate containing cortisol conjugated to horseradish peroxidase and assay diluent) was added to each well using a multichannel pipette. The samples were mixed by placing the microplate on a plate mixer for 5 min before incubation at room temperature for 1 h. Following this, 300 µL of wash buffer (phosphate buffer, detergent and preservative diluted to 1-fold concentration in deionised water) was used to wash each well of the plate four times. After each wash, the plate was carefully blotted on a paper towel before being turned upright. Following that, 200 µL of Tetramethylbenzidine substrate solution was added to each well using a multichannel pipette, mixed on a plate mixer for 5 min and incubated in the dark for 25 min. We then added 50 µL of stop solution and mixed the samples on a plate mixer for 3 min. A microplate reader (Ensight® Multimode Plate Reader, Perkin Elmer; Shelton, CT, USA; Kaleido v3.0 software) was used to read the absorbance at 450 nm within 10 min after introduction of the stop solution. The amount of cortisol enzyme conjugate detected in the sample is inversely proportional to the amount of cortisol found in the subject's sample.

### 2.6. Statistical Analysis

A statistical analysis was performed using SPSS (IBM, version 27.0, Armonk, NY, USA). Visual inspections of histograms and the Shapiro–Wilk test were used to assess the normality of outcomes (Wilcoxon (W) statistic reported). For all outcomes, normally distributed data are presented as the mean ± standard deviation (M ± SD); non-normally distributed data are presented as the median ± inter-quartile range (Mdn ± IQR), unless otherwise specified.

Regarding 2QT, data for 'calming', 'safe' and 'reassuring' were transformed such that a higher score on all adjectives indicated a higher level of threat. Adjectives pertaining to the threat and non-threat rooms were assessed for internal consistency to determine whether the adjective scores could be combined into an overall 2QT score; Cronbach's alpha ($\alpha$) was used to express internal consistency, with a minimum acceptability threshold of 0.69 [32]. Calculation of the 2QT scores was performed using the sum of each statement's transformed rank (1 = Disagree, 2 = Disagree, 3 = Neutral, 4 = Agree, 5 = Agree). In the threat condition, responses to four of the adjectives were not normally distributed ('calming' [W = 0.74, $p$ = 0.001], 'stressful' [W = 0.84, $p$ = 0.02], 'unpleasant' [W = 0.75, $p$ = 0.002] and 'reassuring' [W = 0.81, $p$ = 0.008]), while the responses to 'safe' (W = 0.93, $p$ = 0.356) and 'threatening' (W = 0.90, $p$ = 0.116) were normally distributed. None of the responses in the non-threat condition were normally distributed ('threatening' [W = 0.75, $p$ = 0.002], 'calming' [W = 0.68, $p < 0.001$], 'safe' [W = 0.77, $p$ = 0.003], 'stressful' [W = 0.81, $p$ = 0.009], 'unpleasant' [W = 0.73, $p$ = 0.001] and 'reassuring' [W = 0.83, $p$ = 0.014]). The Mann–Whitney U test (MWU) was performed for the between-group comparisons of 2QT data; the test variables were the combined threat scores, with the condition ('threat' or 'non-threat') as the grouping variable (Mann–Whitney (U) statistic and Z score (Z) reported). The effect size was calculated as $Z/\sqrt{n}$, where Z = Z value of MWU test and $n$ = observation number, and was interpreted as follows: 0.2, small effect; 0.5, medium effect; 0.8, large effect [33].

The cortisol concentration in the threat condition at all 3 timepoints did not follow a normal distribution (cortisol0 [W = 0.81, $p$= 0.012], cortisol20 [W = 0.78, $p$ = 0.006], cortisol26 [W = 0.81, $p$ = 0.012]). The cortisol concentration in the non-threat condition was normally distributed at all three timepoints (cortisol0 [W = 0.97, $p$ = 0.938], cortisol20 [W = 0.92, $p$ = 0.240], cortisol26 [W = 0.93, $p$ = 0.324]). Analyses of within-group cortisol concentrations determined differences across time (cortisol0, cortisol20 and cortisol26). The threat condition was analysed with the Friedman test with Dunn's pairwise post hoc tests and Bonferroni correction; the non-threat condition was analysed with a one-way repeated-measures analysis of variance (ANOVA; cortisol0, cortisol20 and cortisol26 as within-subject factors). An MWU test was used to assess between-group differences.

## 3. Results

### 3.1. Subjects

Twenty-six subjects were recruited (thirteen males, thirteen females) and randomly allocated into either the threat ($n$ = 13; Mdn = 22 ± 8 years) or the non-threat VR condition ($n$ = 13; Mdn = 22 ± 4 years). All subjects' questionnaire data were included; however, one subject (female) exposed to the threat VR condition was excluded from the cortisol analysis due to outlying ELISA results. There were no reported adverse effects of VR for any subject.

### 3.2. Subjective Threat Measurement: 2QT

Internal consistency was deemed acceptable in both the threat ($\alpha$ = 0.88) and the non-threat ($\alpha$ = 0.74) 2QT responses; as such, statement responses were summed for an overall 2QT score for each condition. Subjects in the threat condition recorded a significantly higher 2QT score than those in the non-threat condition (threat Mdn = 22/30 ± 6.5, non-threat Mdn = 10/30 ± 3.5; U = 9.5, Z = −3.86, $p < 0.001$; effect size = 0.76). Adjectives that were associated with negative emotional states (i.e., threatening, stressful, unpleasant) were ranked higher in the threat condition than the non-threat condition ('threatening': U = 35.00, Z = −2.66, $p = 0.008$, effect size = 0.52; 'stressful': U = 19.5, Z = −3.44, $p < 0.001$, effect size = 0.68; 'unpleasant': U = 10.00, Z = −4.01, $p < 0.001$, effect size = 0.79). Adjectives associated with positive emotional states (i.e., calming, safe, reassuring), were ranked higher in the non-threat condition than the threat condition ('calming': U = 13.5, Z = −3.80, $p < 0.001$, effect size = 0.75; 'safe': U = 20.5, Z = −3.39, $p < 0.001$, effect size = 0.67; 'reassuring': U = 9.00, Z = −3.97, $p < 0.001$, effect size = 0.78) (Table 1).

**Table 1.** Subjects' perceptions of threat associated with two clinical virtual reality environments.

| Adjective | Threatening Room ($n$ = 13) | | | | | | Non-Threatening Room ($n$ = 13) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SD | D | N | A | SA | Mdn | SD | D | N | A | SA | Mdn | $p$-Value |
| | (%) | (%) | (%) | (%) | (%) | (IQR) | (%) | (%) | (%) | (%) | (%) | (IQR) | MWU |
| Threatening ^ | 2 (15) | 4 (31) | 3 (23) | 4 (31) | 0 (0) | 3 (2) | 7 (54) | 5 (38) | 1 (8) | 0 (0) | 0 (0) | 1 (1) | $p = 0.008$ * |
| Stressful ^ | 1 (8) | 1 (8) | 2 (15) | 7 (54) | 2 (15) | 4 (1) | 4 (31) | 7 (54) | 2 (15) | 0 (0) | 0 (0) | 2 (1) | $p < 0.001$ * |
| Unpleasant ^ | 0 (0) | 1 (8) | 1 (8) | 9 (69) | 2 (15) | 4 (0) | 5 (38) | 7 (54) | 0 (0) | 1 (8) | 0 (0) | 2 (1) | $p < 0.001$ * |
| Calming # | 5 (38) | 6 (46) | 1 (8) | 0 (0) | 1 (8) | 2 (1) | 0 (0) | 0 (0) | 2 (15) | 10 (77) | 1 (8) | 4 (0) | $p < 0.001$ * |
| Safe # | 0 (0) | 5 (38) | 5 (38) | 2 (15) | 1 (8) | 3 (2) | 0 (0) | 0 (0) | 1 (8) | 6 (46) | 6 (46) | 4 (1) | $p < 0.001$ * |
| Reassuring # | 4 (31) | 7 (54) | 1 (8) | 1 (8) | 0 (0) | 2 (1) | 0 (0) | 0 (0) | 4 (31) | 6 (46) | 3 (23) | 4 (2) | $p < 0.001$ * |
| Cronbach's Alpha | $\alpha$ = 0.88 | | | | | | $\alpha$ = 0.74 | | | | | | |
| Median Threat Score + | 22/30 | | | | | | 10/30 | | | | | | $p < 0.05$ |

^ Threat-associated and # non-threat-associated adjectives, measured on a five-point Likert scale. Frequency percentages are reported for each statement. $n$ = 26 (13 vs. 13) subjects. SD—Strongly Disagree, D—Disagree, N—Neither Agree nor Disagree, A—Agree, SA—Strongly Agree. Mdn (IQR) presented for all adjectives; however, responses to 'safe' and 'threatening' in the Threat condition only were normally distributed. * Statistically significant difference calculated using Mann–Whitney U test (MWU). + Threat scores are summed scores including transformed values.

### 3.3. Physiological Threat Measurement: Cortisol Concentration

There was a significant difference in the cortisol concentration measured across time in the subjects exposed to the threat condition ($\chi^2(2) = 13.83$, $p < 0.001$). Post hoc comparisons revealed a significant decrease in the cortisol concentration from pre-VR exposure to 20 min post-exposure (cortisol0 Mdn = 0.15 ± 0.24 versus cortisol20 Mdn = 0.1 ± 0.2; $p = 0.001$

Bonferroni-corrected). There was no significant difference between the cortisol concentrations at timepoints 20 and 26 min post-VR exposure (cortisol20 Mdn = 0.1 ± 0.2 versus cortisol26 Mdn = 0.12 ± 0.2; $p = 0.922$), but there was a significant decrease from pre-VR exposure to 26 min following exposure (cortisol0 Mdn = 0.15 ± 0.24; cortisol26 Mdn = 0.12 ± 0.2; $p = 0.03$; Figure 3).

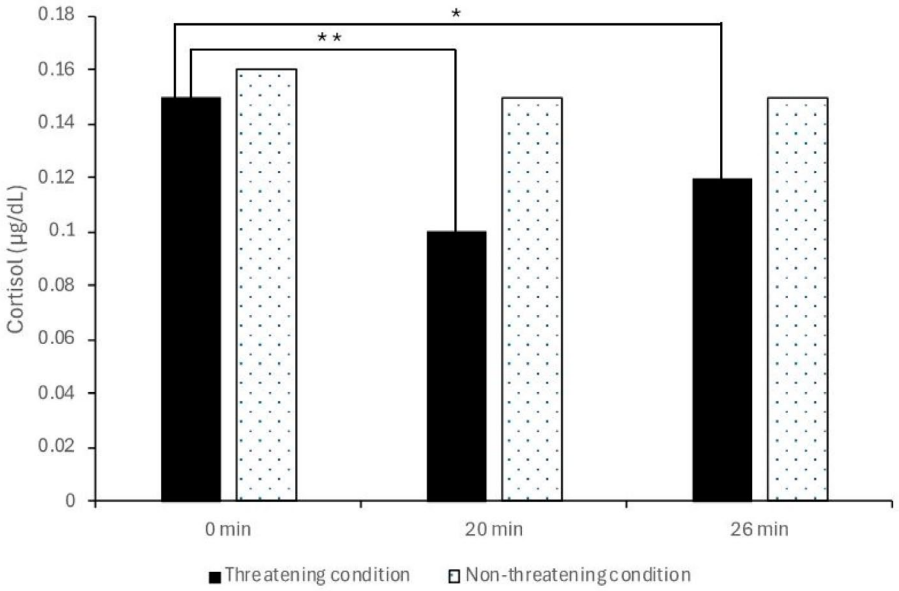



**Figure 3.** Cortisol concentrations in both the threatening and non-threatening conditions at 0 min (pre-virtual reality (VR) exposure) and at 20 min and 26 min post-VR exposure. Cortisol expressed in micrograms per decilitre; all values are medians. Note the significant decrease in cortisol concentration in the threatening condition at both 20 min and 26 min post-VR exposure. No significant change in cortisol was seen in the non-threatening group. * $p < 0.05$ corrected; ** $p = 0.001$ corrected.

There was no significant difference in cortisol found across time in the non-threat condition (cortisol0 M = 0.18 ± 0.09, cortisol20 M = 0.14 ± 0.05, cortisol26 M = 0.17 ± 0.09; $F(2,24) = 1.68$, $p = 0.2$), indicating that cortisol did not change with VR exposure (Figure 3). A between-group comparison revealed no statistically significant difference between the cortisol concentrations of the threat and non-threat conditions at any timepoint (cortisol0 [$U = 91.00$, $Z = -0.71$, $p = 0.50$], cortisol20 [$U = 70.00$, $Z = -0.44$, $p = 0.69$], cortisol26 [$U = 72.00$, $Z = -0.33$, $p = 0.77$]).

## 4. Discussion

The primary of aim of this study was to validate two custom-designed VR clinical consultation rooms for their threat value, using a mixed methods approach. The 2QT results indicated that subjects experienced greater threat in the VR room that was designed and hypothesised to be threatening. Subjects in the threat group recorded significantly higher survey scores than those in the non-threat group in the 2QT, and they more highly ranked the negative adjectives 'threatening', 'stressful' and 'unpleasant'. Subjects allocated to the non-threat VR room found their environment to be less threatening and more strongly associated with the adjectives 'calming', 'safe' and 'reassuring'. Within the threat group, there was a change in the cortisol concentration over time, albeit in a direction we did not anticipate. There was no change in cortisol over time in the non-threat group, nor was there a statistically significant difference in cortisol concentration change between the two conditions.

The most immediate clinical application of the VR conditions established here would be to use the non-threatening setting to provide comfort and safety to someone in acute pain or discomfort, for example, when performing painful joint mobilisation exercises early after a surgical joint replacement. To date, VR has typically been employed as a distraction,

immersing subjects in natural calming environments such as beaches for patients with fibromyalgia [34] or forests and mountains for (unspecified) chronic pain patients [35], reducing pain intensity during VR immersion to varying degrees. Alternatively, VR games involving ice and throwing virtual snowballs have been shown to reduce perceived pain intensity in chronic [36] and acute pain [37] (see [1] for a review). We propose that VR immersion of chronic pain patients in realistic settings, e.g., in nature or, indeed, in a pleasant clinical environment like the one tested here, is potentially more suitable than distraction via gaming. Short-term distraction is unlikely to pose a sustainable long-term solution for chronic (i.e., long-term) pain conditions [38].

Whether or not someone feels threatened in any given situation is subjective, and as such, it was an important aim of this study to determine the subjective experience of threat in the VR clinical rooms. In comparing the endorsed items across the two conditions, the moderate-to-large effect sizes indicate that the design of the threatening VR condition did elicit the feelings of threat and stress as intended. Pain is an entirely subjective experience [28]; in research studies and in the clinic, we rely on someone's self-report [39] (for instance, rating their pain out of 10). Now that this investigation has provided some confirmation of the subjective threatening nature of the VR threat condition, the tool can be used in pain experiments and holds potential to help us understand what goes into someone's pain report.

Our finding of a significant reduction in cortisol from baseline to post-VR exposure in the threat condition is intriguing. One would assume an elevation in cortisol following a threatening stimulus [40], if there were to be any change. It is, of course, possible that the threat was adequate for the subjects to perceive it as threatening but not adequate to result in an increase in salivary cortisol. Past VR studies of threat have employed more stressful or threatening stimuli, for instance, a fear of heights [41], or socio-evaluative contexts such as the Trier Social Stress Test (TSST, a psychosocial stressor shown to elevate acute cortisol levels [42]). The VR conditions in the current study were intended to be realistic clinical settings and, as such, may not have been adequately provocative for a physiological reaction in the hypothalamic–pituitary–adrenal (HPA) axis. However, it is notable that there was no change in cortisol concentration after exposure to the non-threatening condition, which we designed to be comparable apart from the threat value itself. This leads us to propose that there was an effect on the HPA axis release of cortisol, even if the direction was unexpected or at least difficult to explain.

It is possible that the timepoints chosen for post-VR cortisol sampling did not capture the expected stress response. Repeated measures of salivary cortisol are certainly advised to profile the concentration [43]. We based our testing times on a cortisol study that used a VR height challenge [13] and on an overview of the determinants of salivary cortisol responses to acute stress, although that review did recommend measuring at 15 to 20 min post-exposure [44]; hence, it is possible that the cortisol concentration in the threat condition peaked before 20 min. It has been recommended that to investigate the reactivity of the HPA axis to an acute stimulus, saliva should be collected before the stressor and at 15, 30, 45 and 60 min post-stressor. This allows for an evaluation not only of the stress response but also of recovery [45]. There is evidence from animal research to suggest that a rapid uptake of cortisol into cells could explain the apparent decrease we observed in the current investigation [46]. It is possible that 20 min after exposure was too late to capture marked increases in the cortisol concentration; if intracellular uptake occurred prior to sampling, no acute increase would be detected. Salivary cortisol concentrations reflect free plasma cortisol and cannot account for the fraction that is bound or stored intracellularly [46,47]. Interestingly, our findings somewhat align with those of an investigation of reported stress and salivary cortisol concentrations in students under (natural) examination stress. That study also reported that salivary cortisol and perceived stress were inversely correlated, indicating that the relationship is not altogether clear [48].

The cortisol reduction we observed in the threat condition is unlikely to represent the expected decrease in cortisol according to the natural circadian rhythm [49] or potentially

elevated stress levels in subjects on arrival to their study session (i.e., at cortisol0); if either of these explained the reduction, we would have observed a cortisol change in both groups. It is a strength of the study design that subjects were randomised into the two groups and were tested in the same physical environment, so it is doubtful that a hormonal difference existed at baseline. It is hard to argue with the subjects' own reported feelings within the VR context, even if the 2QT used was not a validated questionnaire. The significant difference between the groups in the subjective 2QT responses lends strength to the study findings, as does the demonstrated internal consistency of the scale.

Some limitations of the current work need to be acknowledged. We did not meet our projected sample size and, thus, it is possible we were underpowered to detect between-group differences in cortisol levels. The findings from the current sample, predominantly people aged around 25 years, may not be generalisable to an older population [50], though this has come under debate [51,52]. We would have more closely controlled for confounders with a within-subject (counter-balanced or randomised) design. However, it would be challenging to facilitate such study sessions. With repeated VR exposure and salivary cortisol sampling, the sessions could potentially become very lengthy and tedious, and there is a reported risk of response sensitisation across testing sessions [53]. While the large difference in questionnaire responses would indicate that we did achieve a threatening VR clinical setting, it would be interesting to study the extent to which subjects felt truly immersed or present in their environment. Further investigations conducted with a more sophisticated VR setup might provoke a stronger hormonal change.

Aside from salivary cortisol, there are many other physiological markers of stress. Future explorations and validations of stressful clinical VR environments could sample the heart rate (or heart rate variability), galvanic skin conductance or blood pressure, to name a few. The challenge would be to record these measurements untethered or at least with a minimally obstructive physical setup, to avoid jeopardising the immersion of subjects in the VR and ensure accurate physiological measurement. To more closely explore the threatening nature of the clinical setting, future investigations could incorporate eye-tracking into the VR; the most salient visual features of the environment could potentially be elucidated [54].

Increasingly, VR offers a relatively inexpensive and expedited way of providing a setting that is physically conducive to reducing pain, aiding recovery and promoting healing [55]. While there is only a direct clinical application for the non-threatening condition we report on, importantly, this investigation helped to confirm that we now have customised clinical VR environments that we can deploy and build on in our lab's ongoing investigations of threat as a key factor in someone's experience of pain. By immersing (healthy) subjects into such VR environments while they experience experimental pain, we hope we will have a better understanding of the basic mechanisms of pain, threat being one key feature. Ultimately, for the field of pain, the probing of such questions will mean that the use of VR is more strongly grounded in the evidence and, as such, will potentially offer exciting new avenues for the management of pain.

**Author Contributions:** Conceptualisation, M.T., E.P. and F.D.P.; methodology, D.O., M.T., W.L., W.H.T., E.P. and F.D.P.; recruitment and data collection, D.O., M.T., W.L. and W.H.T.; formal analysis, D.O., M.T., E.P. and F.D.P.; resources, W.L. and F.D.P.; data curation, D.O., M.T., W.L., W.H.T., E.P. and F.D.P.; writing—original draft preparation, D.O., M.T., W.L., E.P. and F.D.P.; writing—review and editing, D.O., M.T., W.L., W.H.T., E.P. and F.D.P.; supervision, M.T., W.L., E.P. and F.D.P.; project administration, M.T. and F.D.P.; funding acquisition, M.T. and F.D.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by Curtin Medical School and the Curtin Hub for Immersive Visualisation and eResearch (HIVE) Summer Internship program (2022–2023).

**Institutional Review Board Statement:** The study was conducted in accordance with the Declaration of Helsinki and approved by the Human Research Ethics Committee of Curtin University (HRE2023-0226; approved 9 May 2023).

**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author due to specifications outlined by the Curtin University Human Research Ethics Committee approval.

**Acknowledgments:** The authors wish to acknowledge the Curtin Hub for Immersive Visualisation and eResearch (HIVE); Rob Steuart and the Curtin Health Innovation Research Institute (CHIRI); Amanda Harders, Donelle Donnelly and the Curtin Health and Wellness Centre; Amy Woodfield; Giuseppe Verdile; Alex Myszka; Wendy Northrop; and Matthew Kenrick.

**Conflicts of Interest:** F.D.P. receives royalties from Elsevier Publishing and Wiley Publishing (textbook authorship). Other authors have no conflicts of interest to declare.

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