Ecological validity of biomarkers in drug research

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CHAPTER 5

VIRTUAL REALITY IN A NOCICEPTIVE PAIN TEST BATTERY: A RANDOMIZED, PLACEBO CONTROLLED TWO-WAY CROSSOVER STUDY WITH DIAZEPAM

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ABSTRACT

Pain is a complex experience influenced by many psychological factors such as emotion, mood, time of day, and stress. We developed a virtual reality pain task that can modulate pain experience, providing possible biomarkers for the affective component in healthy volunteers. Diazepam, a benzodiazepine used for anxiety, may affect this component. We hypothesize that VR-PainCart can assess drug effects on the affective component of pain.

In a randomized crossover study with 24 healthy male participants, we evaluated the effect of a simulated wound in VR on electrical pain detection (PDT) and tolerance (PTT) thresholds during an electrical pain task. Participants underwent pre-dose tests, followed by 5 mg diazepam or placebo, and six rounds of post-dose tests. Each round included an electrical pain test and two VR conditions: (1) VR-wound that increases with stimulus intensity, and (2) VR-neutral: no additional aspects. PDT and PTT were recorded during both VR conditions and without VR. VAS-Questionnaires assessed pain intensity and unpleasantness, and the McGill Pain Questionnaire (MPQ) investigated pain characteristics.

Diazepam increased PDT in the VR-wound environment (ED: 6.0%, CI 2.4–53.2, $p<0.05$). A trend in PTT increase with diazepam in VR-wound was observed but not statistically significant (ED: 6.5%, CI -3.1–17.0%, $p=0.179$). VAS pain intensity and unpleasantness differences between diazepam and placebo were not significantly different.

VR simulated wound enhanced pain perception in an electrical nociceptive task. Diazepam increased PDT in VR-wound, indicating pharmacological modulation of the affective pain component. Future research will include diverse populations and drugs targeting the affective component, such as antidepressants, to evaluate new analgesic compounds.

INTRODUCTION

Pain is complex and cannot be exclusively defined by its intensity. The affective-motivational model suggests that pain includes not only the well-known nociceptive component but also emotional and cognitive dimensions that shape the pain experience.[1] This is consistent with the existence of drugs that, while not directly affecting nociception, still offer analgesic effects due to their anxiolytic or antidepressant properties. Precise pharmacodynamic biomarkers are crucial for determining proof-of-pharmacology, target engagement, and possible efficacy.[2] However, effective biomarkers that quantify the contribution of emotional aspects to pain remain unavailable. Current patient-reported outcome measures (PROMS) that assess the emotional dimension of pain fall short in terms of content validity and psychometric accuracy.[3] Therefore, developing biomarkers that can accurately evaluate this emotional component—commonly referred to as the affective dimension of pain—is of significant interest.

Human pain models are an important tool for evaluating the analgesic effects of drugs and gaining insights into the mechanisms of pain. Nonetheless, no single experimental model can fully capture the complexity of clinical pain.[4] The ‘PainCart®’ contains several sensitive and specific tests for measuring different modalities of nociception and is developed to test analgesics in healthy participants. During testing, the emotional processing of pain is minimized as much as possible by using a standardized silent room with no distractions or interactions.[5] This approach results in a nociceptive test battery with high repeatability and sensitivity to drug concentration effects.[6] However, due to limited emotional processing included in the pain tasks, results may not reflect effects on the inherently subjective affective component of pain.[7] As a result, drugs influencing this component may show no or underestimated effects on this nociceptive test battery.

In a previous study, the painful experience of an electrical stimulation task was successfully modulated using Virtual Reality (VR).[8] During an electrical stimulation task, a simulated wound was presented at the location of the electrodes via a VR-headset. The virtual wound increased in severity with the increase of the stimulus. When comparing the response to this ‘enhanced stimulation’ task to a neutral VR pain task (i.e., without wound), healthy participants had 1) decreased pain detection thresholds, 2) increased perception of VAS pain intensity, and 3) increased perception of VAS pain unpleasantness.

In this study, we administered diazepam, an anxiolytic drug that binds to the GABA-A receptor, increasing the affinity of the receptor and enhancing GABA'S inhibitory effects. The anterior cingulate cortex (ACC) is sensitive to changes in the GABA system and plays a crucial role in pain experience.[9,10] Effects on cerebral blood flow into the temporal regions can already be detected after a single dose of diazepam, and higher pain threshold to a cold pressor test. Additionally, studies have found that a low dose of diazepam influences emotional processing, with limited side effects that might influence study execution (e.g., dizziness or headache)[11] other than a decrease in anxiety.[12]

To assess the sensitivity of the VR pain model to quantify the effects of a pharmacological intervention, a single dose of diazepam (5 mg) was used to reduce emotional processing and the affective pain component.

METHODS

This was a randomized, double-blind, placebo-controlled, two-way cross-over study in healthy participants. The study was conducted in accordance with the Declaration of Helsinki and approved by the local ethical committee (Stichting BEBO, Assen, The Netherlands), and all participants provided written informed consent prior to any study related activities. Before commencing the study visits, participants were medically screened during a separate visit and when found eligible they were included in the study. Participants received a single dose of diazepam 5 mg and matching placebo in randomized order on two separate study visits. Drug administrations were separated by a washout period of 7 days. VR-PainCart assessments were performed twice pre-dose and repeated hourly up to 6 h after drug administration. Participants were required to fast (only allowed to drink water) 2 hours prior to drug administration up to 1.5 hours post-dose. Drug administration occurred in the morning between 10:00 and 11:30 for all participants after which relative mealtimes were standardized. No blood samples were collected to analyse diazepam serum concentrations due to its well-known pharmacokinetic parameters. A follow-up phone-call was performed 7 to 10 days after the last drug administration to record any adverse events and medication usage. See Figure S1 for a schematic overview of the study design.

Participants

Healthy male participants aged 23 to 35 were enrolled in the study. To ensure avatar realism in the VR simulation, only participants with light to medium skin tones (i.e., Fitzpatrick scale ≤ IV) were included. Additionally, participants were required to have no skin deformations or discolorations on their upper and lower limbs. Eligibility was further restricted to those with a pain tolerance threshold below 80% of the maximum stimulation in the test conducted without VR, any history of psychiatric illness or vision disorders, and a history of simulator sickness based on previous experience in VR or other simulators. Additionally, participants who smoked more than 5 cigarettes per day or consumed more than 8 units of (methyl)-xanthines daily were excluded due to potential withdrawal symptoms during the study periods and to reduce possible effects on pain thresholds.[13] During the screening process, participants were not trained on or informed about the contents of the VR simulation.

Assessments

All assessments were performed in a quiet room with controlled lightning and temperature. During all assessments only the participant and the research assistants were present in the room. Materials and procedures of the electrical stair test and VR enhancement were identical to the version used in the previous study.[8]

Electrical stair test

The electrical stair test[14] used two AG-AGCL electrodes placed on the tibial bone to evaluate cutaneous electrical pain. Single electrical stimuli, each lasting 0.2 ms, were administered, starting at 0 mA and incrementing by 0.5 mA up to a maximum of 50 mA. Participants were provided with an electronic Visual Analog Scale (EVAS) and instructed to move the slider as soon as the stimulus became painful. The intensity at which pain is first detected, is defined as the pain detection threshold (PDT), and the first endpoint of this assessment. The second endpoint, the pain tolerance threshold (PTT), is recorded when the participant indicates the maximum value on the EVAS, representing the highest level of pain they can tolerate. If the PTT is not reached before 50 mA, the test automatically stops after a maximum total duration of 120 seconds.

Virtual Reality

MATERIALS During the pain assessments that included VR, participants wore a VR headset with headphones (Vive Pro, HTC). The VR environment simulated the room in which participants performed all assessments including an avatar in the same sitting position which was viewed from first-person perspective (see Figure S2). Avatar size could be adjusted according to the height of the participant. All equipment needed for the electrical stair pain test is included in the simulation, including the stimulator, electrodes on the leg, and an EVAS slider. The position of the legs, hands, and VAS slider were tracked using HTC VIVE trackers and a leap motion sensor.

Prior to each VR assessment, participants were primed by performing a set of instructions encouraging interaction with the VR environment. The instructions included asking the participant to grab the VAS slider from the sky (handed to them by the assistant) and describe objects located in the room.

VR CONDITIONS There were two different VR conditions: (1) VR-Wound and (2) VR-Neutral. The VR-Wound condition showed the progressive development of a burn wound with blood, burned skin, and smoke, around the leg electrodes (see Figure 1). The intensity of the wound increased simultaneously with the intensity of the pain test. This simulation was accompanied by sounds of electrical sparks and sizzling noises through the VR headset. The simulation started directly at the beginning of the test and reached maximum intensity at 40 seconds. After 40 seconds, the intensity of the audio-visual simulation no longer increased but continued until the test is stopped. This duration was chosen to make sure most of the participants experience the full simulation. During the VR-Neutral condition, no additional visual or auditory stimulations were applied.

SUBJECTIVE EXPERIENCE OF PAIN AND VR Subjective pain experience was assessed after each pain test including VR by the McGill Short Form[15] and two visual analogue scales (VAS) for the unpleasantness and intensity of pain. Additionally, after the VR-Wound condition, three VAS questions evaluating the (1) focus on the wound, (2) realism, and (3) unpleasantness of the wound were assessed.

EMBODIMENT After each assessment including VR, the level of embodiment was recorded with the embodiment questionnaire including 6 items on a 7-point Likert scale (1: completely disagree, 7: completely agree). The questions each focussed on a different aspect of the embodiment of the virtual body: ownership of body parts, ownership of the body, wound as part of the body (only after VR-Wound simulation), ownership of movement, control of the virtual body, illusion of another body.

Analysis

This is an exploratory study; therefore, the sample size is not based on a sample size calculation. The sample size is the same as the previous study which showed significant effects of the VR-Wound simulation with 24 participants. Statistical analyses were performed using the SAS Version 9.4 (SAS Institute INC., Cary, NC, USA)

Each parameter was analysed with a mixed-model analysis of covariance with treatment, period, condition (if applicable), time and treatment by time, condition by time (if applicable) and treatment by condition by time (if applicable), random factors participant, participant by treatment and participant by time and the average prevalue as covariate.

For wound specific parameters (VAS focus, VAS realism, and VAS unpleasantness of the wound), the VR setting effect and its interactions are not calculated since there is only one VR-Wound setting per timepoint and there are no degrees of freedom left.

The Kenward-Roger approximation was used to estimate denominator degrees of freedom, and model parameters were estimated using the restricted maximum likelihood method.

The general treatment effect and specific contrasts were reported with the estimated difference and the 95% CI, the least square mean (LSM) estimates, and the p-value. Graphs of the LSM estimates over time by treatment were presented with 95% CI as error bars and change from baseline LSM estimates.

The following contrasts are calculated within the models: Diazepam – Placebo. And where applicable: Diazepam – Placebo within no VR; Diazepam – Placebo within VR-Neutral; Diazepam – Placebo within VR-Wound; Diazepam – Placebo within VR-Neutral as first; Diazepam – Placebo within VR-Neutral as second; Diazepam – Placebo within VR-Wound as first; Diazepam – Placebo within VR-Wound as second. For the electric stair PDT and PTT, also: VR-Neutral – no VR within Placebo; VR-Wound – VR-Neutral within Placebo; VR-Neutral – no VR within Diazepam; VR-Wound – VR-Neutral within Diazepam. The results were not corrected for multiple testing.

RESULTS

Participants

A total of 24 healthy male participants were enrolled in the study. None of the participants discontinued participation or were excluded from the analysis (age mean (SD) is 22.0 (2.4), range 18 – 28, and BMI of 23.6 kg/m^2 (2.8), range 19.8 – 29.3). Due to a technical error, VR-Neutral simulation data of the first visit for two participants was lost. Few adverse events (AE) were recorded. All AEs were mild, transient and confirm the known effects of diazepam at this dose level.

Pain thresholds

Table 1 presents the least square means of pain thresholds derived from the statistical model, along with the contrasts between placebo and diazepam. Additional contrasts within treatments are provided in Table 2.

Diazepam vs placebo

Compared to placebo, diazepam significantly increased the PDT for the VR-Wound condition (ED = 25.2%, 95% CI: 2.4 to 53.2, p = .030). However, diazepam did not significantly affect the PDT for the pain task outside VR (ED = -3.6%, 95% CI: -21.2 to 18.0, p = .715) or the neutral VR simulation (ED = -1.3%, 95% CI: -19.3 to 20.7, p = .897) (See Figure 2). Diazepam also did not significantly affect the PTT in any of the conditions.

Effects of VR on pain thresholds

During the placebo study period, the neutral VR simulation had no significant effect on the PDT or PTT compared to no VR (PDT: ED = 6.9%, 95% CI: -1.8 to 16.3, p = .123; PTT: ED = 1.7%, 95% CI: -0.3 to 3.7, p = .089) (see Table 2). However, in the diazepam study period, the neutral VR simulation significantly increased both PDT and PTT compared to the pain task outside of VR (PDT: ED = 9.4%, 95% CI: 0.6 to 19.1, p = .037; PTT: ED = 2.1%, 95% CI: 0.2 to 4.2, p = .033) (see Table 1).

When comparing the VR-Wound condition to the VR-Neutral condition, we observed a significant decrease in PDT during the placebo period (ED = -13.8%, 95% CI: -20.7 to -6.3, p < .001) (see Table 2). In contrast, during the diazepam study period, the VR-wound condition significantly increased PDT (ED = 9.4%, 95% CI: 0.5 to 19.0, p = .037). No effect on PTT was observed during the placebo period, but following diazepam administration, PTT was significantly increased in the VR-Wound condition compared to the VR-Neutral condition (ED = 2.9%, 95% CI: 1.0 to 4.9, p = .003).

Questionnaires

There were no significant differences in VAS ratings for unpleasantness or intensity across any of the VR conditions or treatment effects. The VAS ratings for focus, realism, and unpleasantness of the wound were similar between the two treatments. The McGill questionnaire showed no treatment effects for either the total score or the subdomains (sensory, affective, present pain intensity, and pain score).

All questions related to the level of embodiment remained relatively stable across assessments and treatments, except for one item. The question assessing the feeling of movement control of the virtual body showed a significant increase for diazepam compared to placebo.

DISCUSSION

This study is the first to demonstrate that augmentation of the pain experience induced by an enhanced virtual reality simulation that was integrated into a nociceptive pain test battery, can be attenuated using an anxiolytic drug. Here we demonstrated that this reduction in PDT was significantly attenuated when the participant received a single oral dose of diazepam. In fact, the PDT in the VR-Wound condition was significantly higher than the PDT in the neutral VR condition. The VR-PainCart successfully isolated the affective pain component from changes in nociception, as virtual reality raised the pain detection threshold but did not affect the pain tolerance threshold after administration of diazepam. With this study, we replicated the previous findings that the addition of a wound in VR on the location of the painful stimuli significantly decreased the PDT compared to not presenting this wound. Additionally, we reproduce previous findings where a neutral VR simulation increased the PDT, but not the PTT.[8]

The administration of diazepam significantly mitigated the reduction in pain detection threshold to electrical pain caused by the VR-Wound simulation (See Figure 2). This finding not only confirms that diazepam alters the pain experience and can be demonstrated to have analgesic properties in this model. Additionally, the lack of effect on the other two VR conditions (no-VR and VR-Neutral) confirms that this effect is isolated from nociception, which was not influenced by diazepam. This isolated effect builds upon the hypothesis of a precious study assessing the effect of diazepam on pain. There, using a pressure cuff on the upper arm concluded that the effect of diazepam should be assigned to the emotional aspect of pain and not a change in nociception.[16]

There was no effect of diazepam or VR on the PTT. Pain-related emotions, cognitive interpretation, and subjective catastrophizing of future consequences can be triggered by the immediate sensory unpleasantness of a pain stimulus.[17] Whilst diazepam could create an emotional disconnect between the two during onset of pain, pain-related emotions during the experience may already be active once the PTT is reached.

Participants did not experience the pain any differently following administration of diazepam as recorded with the different questionnaires, even though the PDT was elevated. This shows that the pain task using the VR-PainCart cannot be replaced with commonly used methods such as the McGill questionnaire, which includes an affective subscale. The lack of change in the McGill short form, also shows that the sensory characteristics of the stimuli are unaffected, maintaining the realism of the stimulus.

The level of embodiment experienced by the participants remained stable and was mostly unaffected by diazepam, except for one parameter: control of movement seemed to be improved by diazepam compared to placebo. One explanation could be related to reduced general motion activity caused by benzodiazepines.[18] Diazepam may also have led to a slight reduction of CNS processing resulting in less observations of the lag time and therefore a sense of better control.

While several significant differences in PDT and PTT were found between different VR condition, no effects were observed on the subjective rating scales for pain intensity and pain unpleasantness. This is contrary to the previous findings in the first VR-PainCart study were participants indicated a higher pain intensity and unpleasantness under the VR-Wound condition.[8] The previously found effects might have been different due to the lack of blinding resulting in socially desirable answers. On the other hand, the number of questionnaires in this study was quite large with the addition of the McGill questionnaire, and as such the increased time between pain task and questionnaire may have influenced the responses. Therefore, selection of the questionnaires might improve reliability.

Two limitations of the study were (1) the relatively small sample size and (2) the inclusion criteria focussed on healthy young men. A backwards power calculation demonstrates that the study was sufficiently powered to detect a difference of 1.076 with a standard deviation 1.27 (results of VR-Wound simulation) in PDT between diazepam and placebo treatment with power of 80% and alpha of 0.05. Because the study was only performed in men, we cannot generalize the results of this study to other populations (e.g., different age, gender, or personality characteristics). It may be possible that healthy women or elderly respond differently to the VR-PainCart and show different modulating effects. The personality characteristics and perhaps different emotional state of (chronic) pain patients[19] make it difficult to predict the modulating effect of the VR-PainCart on their pain experience. Additionally, the response to the VR challenge on the emotional processing might be absent or over-active in different clinical populations, e.g. in patients with pain and central sensitization.[20-22] Future research should include patients to identify the clinical relevance of the biomarker and to provide a predictive validity in early drug development.

The findings of this validation study provide valuable insights into the potential of the PainCart, a pain test battery already known for its high repeatability and sensitivity to nociceptive tests. Now, with the addition of VR simulations targeting the affective aspect of pain, the VR-PainCart addresses the need for precise pharmacodynamic biomarkers that are critical for establishing proof-of-pharmacology or target engagement. This is particularly significant as there is a growing demand for effective new analgesics, though recent efforts in drug discovery have unfortunately not resulted in effective treatment of chronic pain.

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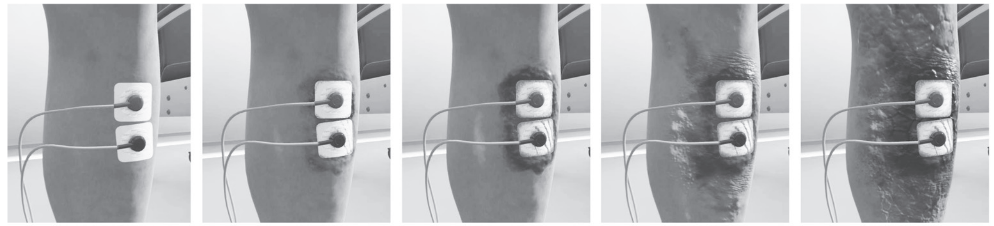
FIGURE 1 The neutral simulation did not include the burn wound (left). During the wound simulation, the wound around the electrodes increased in size and severity (from left to right).



(for color image see page 85)

FIGURE 2 Estimated difference between diazepam and placebo treatment for the Electrical Stair PDT overall, without VR, within VR-Neutral simulation and VR-Wound simulation.

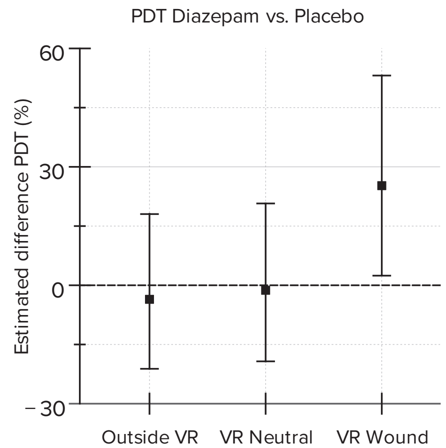


TABLE 1 Pain thresholds recorded in mA for each VR-condition and both treatments including the contrast between placebo and diazepam with the percentage of change in thresholds.

	No VR		VR-Neutral		VR-Wound	
	PDT	PTT	PDT	PTT	PDT	PTT
Placebo (mA)	4.63	19.95	4.95	20.29	4.26	20.08
Diazepam (mA)	4.46	20.34	4.88	20.78	5.34	21.38
Placebo vs diazepam	-3.6% (-21.2; 18.0) p=.715	1.9% (-7.3; 12.0) p=.676	-1.3% (-19.3; 20.7) p=.897	2.4% (-6.8; 12.5) p=.608	25.2% (2.4; 53.2) **p=.030**	6.5% (-3.1; 17.0) **p=.179**

VR: Virtual Reality. PDT: Pain Detection threshold. PTT: Pain Tolerance Threshold. Bold indicates statistical difference (p<.05).

TABLE 2 Statistical contrasts (% of change) within treatment.
VR: Virtual Reality. PDT: Pain Detection Threshold.

Contrast	Placebo		Diazepam	
	PDT	PTT	PDT	PTT
No VR – VR-neutral	6.9% (-1.8; 16.3) p=.123	1.7% (-0.3; 3.7) p=.089	9.4% (0.6; 19.1) **p=.037**	2.1% (0.2; 4.2) **p=.033**
VR-neutral – VR-wound	-13.8% (-20.7; -6.3) **p<.001**	-1.1% (-3.0; 0.9) p=.277	9.4% (0.5; 19.0) **p=.037**	2.9% (1.0; 4.9) **p=.003**

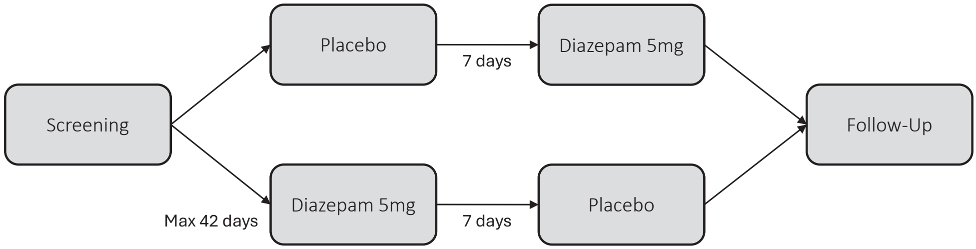
PTT: Pain Tolerance Threshold. Bold indicates statistical difference (p<.05).

SUPPLEMENTS

SUPPLEMENTARY TABEL S1 Overview of adverse events

Summary of Number of Subjects with TEAEs by Treatment, SOC, PT and Severity, Safety population						
		Diazepam (N=24)			Placebo (N=24)	
System Organ Class/ Preferred Term	Mild N	Moderate N	Severe N	Mild N	Moderate N	Severe N
ANY EVENTS	12	-	-	7	-	-
GASTROINTESTINAL DISORDERS	-	-	-	1	-	-
Gastrooesophageal reflux disease	-	-	-	1	-	-
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS	4	-	-	1	-	-
Fatigue	4	-	-	1	-	-
INFECTIONS AND INFESTATIONS	-	-	-	1	-	-
Nasopharyngitis	-	-	-	1	-	-
NERVOUS SYSTEM DISORDERS	7	-	-	4	-	-
Dizziness	1	-	-	-	-	-
Sedation	-	-	-	1	-	-
Somnolence	6	-	-	3	-	-
PSYCHIATRIC DISORDERS	-	-	-	2	-	-
Flat affect	-	-	-	1	-	-
Insomnia	-	-	-	1	-	-

SUPPLEMENT FIGURE S1 Schematic overview of study design



SUPPLEMENT FIGURE S2 View of the participant during the electrical stair assessments with Virtual Reality.