Summary of the research program

Project name	**A study of the effects and mechanisms of virtual reality features on upper limb motor function in stroke patients**
research purpose	Main purpose: To understand the effects of the immersive and interactive nature of VR on upper limb motor function rehabilitation and body representation remodeling in stroke patients and the neural mechanisms underlying their mediated motor function improvement. Exploratory purpose: To explore the differences in rehabilitation effects of different virtual scenarios; to investigate how VR can facilitate the formation of patients' motor intentions and further enhance the recovery and remodeling of upper limb function.
research hypothesis	The combined effect of immersion and interactivity of VR can integrate multisensory inputs and motor outputs, induce a sense of ownership and autonomy, and reshape body representations, which in turn enhances the recovery of upper limb motor function after stroke through neuroplasticity mechanisms.
Research design	Single-center, randomized, single-blind controlled clinical trials
subject population	Inclusion criteria: 1. First episode of stroke, hemorrhagic or ischemic, confirmed by computed tomography or magnetic resonance imaging of the brain; 2. Age 40-80 years, either male or female; 3. 3-12 months since stroke occurrence; 4. Mild-to-moderate or moderate-to-severe upper extremity impairment, with a FMA-UE score between 16 and 53; 5. Basic ability to communicate and comprehend the research instructions, with a score of 21 and above on the MMSE scale; 6. Agreement to participate in the study, with an informed consent form duly signed by the patient or a family member.

	Exclusion Criteria: 1. The presence of ferromagnetic metals implanted in the body; 2. Visual or hearing deficits; 3. Unstable medical conditions; 4. History of receiving similar VR training in the past; 5. A history of myasthenia gravis, multiple sclerosis, muscular dystrophy, or other diseases that may cause upper limb movement disorders.
diagnostic criteria	1. Based on history, symptoms and signs, combined with CT or MRI imaging methods suggestive of ischemic or hemorrhagic stroke; 2. Post-stroke upper extremity motor dysfunction is a manifestation of an individual's impairment in performing motor control and functional use of upper extremity areas such as the arm, forearm, and hand due to stroke-induced brain damage; 3. FMA-UE, ARAT, or NIHSS screening suggestive of upper extremity motor dysfunction.
sample size	This was a randomized controlled study with FMA-UE as the primary outcome indicator, so the sample size was calculated based on the change in FMA-UE scores. Sample size was calculated using analysis of variance for comparisons between multiple group means, and the sample was calculated using the formula n = $\varphi^2\left(\sum S_i^2/g\right)[\sum(\bar{x}_i-\bar{x})^2/(g-1)]$ The number of groups (g) was 3, and the number of participants in the interactive VR group, the immersive and interactive VR group, and the control group was 1:1:1. According to previous studies, the mean of each group was 35.4 35.7 46.5, and the standard deviation (σ) of the difference of each group was 15.3 18 14.2, and the mean standard deviation (σ) was 15.8. Requiring a two-sided test,α is the probability of Type I error, which was set to 0.05; 1 -β is the degree of certainty (test efficacy), set to 80%, so β is 0.2.

	K is the average distribution ratio of the sample size of each group of data, set to 1. According to α, β, v1, v2 look up the table to get the value. Using PASS15 software, the total sample size was calculated to be 96, and the sample size of each group was 32.Considering the 20% dropout rate, 120 patients needed to be included in the final study, and the sample size of each group was 40.
intervening factor	Patient characteristics: e.g., age, gender, underlying disease, etc. Types of interventions: e.g. interactive VR interventions, immersive and interactive VR interventions, etc. Duration and frequency of interventions: how many times per week and duration of each intervention
ending variable	Primary endpoint: FMA-UE (6 weeks) Secondary endpoints: behavioral indicators (Sense of Ownership Questionnaire, proprioceptive drift scale, ARAT, NIHSS, MMSE), electrophysiological indicators (EMG, EEG), imaging indicators (fMRI)
Follow-up program	6 weeks
sample collection	☑No□ Yes Brief description of the type of organization, volume
Unconventional inspections	☑No□ Yes **Such as non-essential CTs, imaging, lab tests, punctures, etc.**
Statistical methods	Measurements of the three groups at three time points were statistically analyzed using SPSS25 software. All measurements were first tested for normality using the Shapiro-Wilk method. Measures that conformed to normal distribution were expressed as mean± standard deviation, and within-group comparisons were made using repeated measures ANOVA, whereby results at post-intervention and follow-up were compared to baseline (initial pre-treatment assessment). In case of statistically significant differences, further post-hoc multiple comparisons will be executed to observe specific differences between time points. Comparisons between groups will be made using one-way ANOVA and further two-way comparisons will also be performed if there are significant differences between groups. In addition, non-normally distributed measures were expressed as medians (upper and lower quartiles), and Friedman's test was

	used for within-group before-and-after comparisons, while Kruska-Walls H test was used for between-group comparisons. Differences were considered statistically significant at $P < 0.05$.
Research risk self-assessment	□High risk studies□ Medium risk studies☑ Low risk studies brief statement In terms of the nature of the intervention, the intervention used in this study (virtual reality training) was non-invasive and safe, with fewer side effects; and in terms of participant selection, the choice of study participants was limited to relatively healthy or medically stable patients, thereby reducing risk.

Reference framework for the detailed research program (if not relevant enter: not applicable or delete)

1. Rationale for the project

1.1 Background of the study:

Stroke is a global health problem and one of the leading causes of death and disability in adults. According to epidemiologic data, approximately 15 million people suffer from stroke each year, of whom approximately 5 million die as a result. 77.4% of stroke survivors experience varying degrees of upper limb dysfunction or even lifelong disability [1]. Although advances in medical technology have led to a reduction in stroke mortality in recent years, the absolute number of stroke cases is increasing with the aging of the population. This trend has greatly increased the social demand for rehabilitation medicine and services [2-3], placing a heavy burden on patients and their families while pushing up social healthcare expenditures.S. Rajsic et al. conducted a systematic review of multiple papers related to post-stroke rehabilitation from 2000-2016 and found that the cost of post-stroke rehabilitation was as high as $4,850/month in the United States [4].

Therefore, it is crucial to develop and implement effective rehabilitation strategies. It is well known that traditional rehabilitation techniques have some limitations, such as lack of fun and effective incentives [5-6], and attempts of emerging technologies are working to change this. Many studies have shown that the neuroplasticity of the brain is the theoretical basis for motor rehabilitation after stroke [7-8]. Therefore, among these attempts, the application of technologies to remodel brain plasticity after stroke has flourished, such as noninvasive brain stimulation techniques such as transcranial magnetic stimulation (TMS) and transcranial direct current stimulation (tDCS), therapies based on mirror neuron systems, rehabilitation robots, and brain-computer interfaces (BCIs). Notably, the advent of virtual reality (VR) technology has also opened up new possibilities for rehabilitation [9]. Post-stroke functional magnetic resonance imaging studies have shown that VR interventions are able to remodel the central nervous system of patients, providing new horizons for breaking through the post-stroke rehabilitation plateau period[10-11].

VR utilizes computer hardware and software tools to build an interactive simulation environment that provides real-time stimulation through multiple sensory channels - visual, auditory, tactile, and even taste and smell - to facilitate natural interaction between embedded objects (user avatars and virtual environments) [12-13]. Based on the three fundamental characteristics of Immersion, Interaction and Imagination, the so-called "3Is", VR is able to create an immersive experience that looks and feels like the real world, and thus provide patients with rich and personalized treatment options for patients. First, "immersion" refers to the physical and psychological sensation that users experience in a virtual environment, which requires active

participation as if they were actually in the virtual world. Secondly, "interactivity" means that users can interact with elements in the virtual environment in real time and dynamically, and the virtual environment can respond to user inputs, which in turn can influence the user and enhance the user's sense of reality in the virtual environment. Finally, "conceptualization" refers to the creative space provided by VR, which allows users to construct new ideas and concepts that cannot be directly perceived in reality, thus stimulating their creativity and encouraging them to explore situations that cannot be realized in the real world [14]. In this way, the "3Is" of VR complement each other to provide users with experiences that are both interesting and responsive to their rehabilitation needs.

1.2 Status of the study:

Currently, many studies have been conducted to investigate the effectiveness of VR intervention in stroke rehabilitation, for example, Qianqian Huang et al. showed that VR training can effectively improve upper limb function in stroke patients [15]; Nasir Sultan and his team found that VR training significantly improved independence, trunk coordination and functional motor ability in stroke patients [16]; and Ana Lúcia Faria et al. pointed out that VR training also had a significant effect on improving cognitive ability in stroke patients [17].]; furthermore, Ana Lúcia Faria et al. showed that VR training also had a significant effect on improving cognitive performance in stroke patients [17]. These studies have focused on evaluating the effects of VR as a holistic intervention, but have neglected the important role that its intrinsic properties play in this process.

Studies have shown that VR plays an important role in post-stroke rehabilitation, and the key lies in its ability to induce a body perception illusion, which is enhanced by the "3I" feature of VR [18]. The Rubber Hand Illusion (RHI) experiment is a classical paradigm for the study of body illusions. In a traditional RHI experiment, the visual attention of the participant is focused on the rubber hand, and then tactile or painful stimuli are applied synchronously to the rubber hand and the participant's hidden real hand, so that visual, tactile, and painful stimulus information is transmitted to the participant's CNS, so that the visually visible rubber hand is incorporated into the participant's bodily representation, causing the patient to have a body perception illusion that "feeling that the rubber hand is like their own" [19].

The immersive and interactive nature of VR is capable of inducing a wide range of illusions from the environment to the body, making VR-based multichannel synergistic stimulation an effective means of remodeling external representations of the brain. For example, in addition to inducing a sense of possession on a robotic or rubber hand, many subsequent studies have tried different "virtual hand illusion" (VHI) experiments, in which the virtual hand is displayed on a screen or in an immersive 3D virtual space [20-22]. These VHI studies not only validate the findings of classical RHI experiments that a sense of possession can be induced in artificial or

virtual hands, but also make the experiments more flexible. For example, the shape of the virtual hand can be dynamically adjusted and can also be moved freely, which is not possible in traditional RHI experiments [23]. As VR technology continues to evolve, more new experimental paradigms for studying the sense of ownership or agency may emerge. However, the corresponding studies on VR characteristics have not been further extended to the practical clinical application of post-stroke rehabilitation [24-25].

2. Purpose of the experiment

2.1 Main research objectives:

To understand the effects of the immersive and interactive nature of VR on upper limb motor function rehabilitation and body representation remodeling in stroke patients and the neural mechanisms underlying their mediated motor function improvement.

2.2 Secondary and exploratory research objectives

To explore the differences in rehabilitation effects of different virtual scenarios; to investigate how VR can facilitate the formation of patients' motor intentions and further enhance the recovery and remodeling of upper limb function.

3. Pilot program

3.1 Experimental design

Single-center, randomized, single-blind controlled clinical trials

3.2 Selection of the study population

3.2.1 Inclusion criteria:

(1) First episode of stroke, hemorrhagic or ischemic, confirmed by computed tomography or magnetic resonance imaging of the brain;

(2) Age 40-80 years, either male or female;

(3) 3-12 months since stroke occurrence;

(4)Mild-to-moderate or moderate-to-severe upper extremity impairment, with a FMA-UE score between 16 and 53;

(5) Basic ability to communicate and comprehend the research instructions, with a score of 21 and above on the MMSE scale;

(6)Agreement to participate in the study, with an informed consent form duly signed by the patient or a family member

3.2.2 .Exclusion criteria:

(1) The presence of a ferromagnetic metal implant in the body;

(2) Visual or hearing deficits;

(3) Unstable medical conditions;

(4) History of receiving similar VR training in the past;

(5) A history of myasthenia gravis, multiple sclerosis, muscular dystrophy, or other diseases that may cause upper limb movement disorders.

3.3 Criteria for participant withdrawal from the trial

(1) Participants requested to be withdrawn from the clinical study;

(2) Those who are unable to complete the relevant checks as required, affecting data collection;

(3) Those who are privately receiving other treatment programs during the treatment period.

3.4 Early termination of tests/closure of test centers

(1) In the event of a serious adverse reaction during treatment, the trial was discontinued at the combined decision of the participant and the investigator;

(2) Significant protocol errors in the trial that make it difficult to evaluate clinical efficacy; or a well-designed protocol, however, significant deviations occurred during implementation;

(3) The participant develops other illnesses or serious complications during treatment that require treatment or resuscitation.

3.5 Treatment

3.5.1 Treatment received by participants

Before the study is conducted, the researcher must provide the participants with the post-test intervention.

Prior to the intervention, we conducted a VHI experiment on all experimental groups to help stroke patients reintegrate their cognition of the hemiplegic side of the upper limb. The specific experimental procedure is described below:

Participants were asked to sit at a table with their hands secured to the tabletop to ensure that they were comfortable and able to maintain this position for an extended period of time, and were given a commercially available HTC Vive head-mounted display (HMD, https://www.vive.com) to wear. The virtual scene presented in the HMD was a darkened room with a table of the same size and color as the real table in the experimental setting, and a realistic virtual hand model visible from the first-person perspective, which was positioned and posed to correspond to the participant's real hand. During the experiment, participants were required to keep their head stationary and their visual orientation constant. Previous studies have demonstrated that the sensitivity of the RHI is independent of hand laterality, so we tested only the affected hand.

We used a 6-degree-of-freedom handle to tap and stroke the participant's real hand, the position of which was tracked by the Intersense 900 system to determine the position of the virtual handle. Thus, when the experimenter moved the handle, the virtual handle was synchronized to reflect the movement. Participants felt the real handle tapping and stroking their hand while seeing the virtual handle doing the same to the virtual hand. This process lasted a total of 5 minutes.

To further enhance haptic feedback, we mounted a small stimulator on the back of the palm of the participant's affected hand to deliver vibrotactile stimuli to their hand. The stimulator was triggered by an Arduino microcontroller (https://www.arduino.cc) via a customized script developed in the Unity software environment (http://unity.com). During the experiment, a virtual ball appears in the virtual scene, which moves up and down according to a preset animation. When the virtual ball reaches the virtual part corresponding to the stimulator position, the stimulator vibrates synchronously for about 500 ms. The stimulation phase lasted 60 seconds and was repeated six times.

Interactive VR group: similar to the VHI experiment, participants were required to wear an HMD and then enter a virtual experimental environment. The VR scenes in this group were based on basic motor interactions and contained a realistic model of the hemiplegic-side virtual hand, a virtual sphere, and a virtual background identical to the real experimental environment, and they were all presented in a first-person view. The program commands the hemiplegic-side virtual hand to perform specific interactive tasks, such as grasping the virtual sphere - holding the virtual sphere for 3 seconds at a time for a duration of 3 seconds and then relaxing it for 3 seconds. At the same time, a real sphere of the same size and color as the virtual ball is also provided in the real world, and the participant is instructed to perform the same movement as synchronously as possible with the real hand on the hemiplegic side. The treatment was conducted four days a week for one hour per day for two weeks.

Immersive and Interactive VR Group: The virtual environments in this group were designed to combine rich immersive VR scenarios with specific interactive tasks. Specifically, there were two virtual environments: (1) Natural Landscape: Participants were on a peaceful forest trail surrounded by tall trees, birdsong, and the sound of rushing water. In this scenario, the program commands the hemiplegic side virtual hand to grasp a spherical fruit on a tree, holding the fruit for 3 seconds at a time for a duration of 3 seconds, followed by 3 seconds of relaxation; (2) City park: the participant is in a city park surrounded by the sounds of children playing and the background sounds of the natural environment. In this scenario, the program causes the hemiplegic side virtual hand to grasp a ping-pong ball in the park, holding it for 3 seconds at a time and then relaxing it for 3 seconds. To enhance the sense of actual participation, a real sphere of the same size and color as the virtual object will be provided in the reality, and participants will be instructed to synchronize the execution of the corresponding movements with the real hand of the hemiplegic side as much as possible. The treatment will take place four days a week for one hour per day for two weeks.

Control group: the VR scene in this group did not have a complex storyline or visual effects and did not emphasize specific interactive tasks. It contains only a first-perspective realistic model of the hemiplegic side virtual hand and a virtual background identical to the real experimental

environment. The program will have the hemiplegic-side virtual hand perform simple and repetitive grasping motions, with each fist clench lasting 3 seconds, followed by 3 seconds of relaxation. Participants were also instructed to try to perform the same movements simultaneously with the real hand on the hemiplegic side. The treatment is performed 4 days a week for 1 hour per day for two weeks.

3.5.2 Participant grouping methodology

One hundred and twenty stroke hemiplegic patients hospitalized in the Department of Rehabilitation Medicine of Qilu Hospital of Shandong University were selected as the research subjects of this experiment. The patients were randomly divided into 3 groups using the method of envelope drawing: interactive VR group, immersive and interactive VR group, and control group, with 40 cases in each group.

3.5.3 Dose selection in the test

The above intervention methods and scenarios of this study are based on references and the clinical practice of our group, which are in accordance with the guidelines for the application of virtual reality, and the occurrence of adverse reactions is also recorded.

3.5.4 Blinding

Due to the nature of the intervention, it was not possible to blind participants and intervention therapists. Therefore, a single-blind method of outcome data assessment and analysts was used.

3.5.5 Treatment Adherence Safeguards

Patient education: provide clear information about the treatment plan, explaining the purpose, process and expected effects of the intervention to help patients understand its importance; Simplify the treatment plan: simplify the treatment process as much as possible, e.g., reduce the number of times medication is taken, use convenient equipment, etc., to reduce the patient's burden; Incentives: consider creating incentives, e.g., encourage patients to adhere to the treatment by providing small rewards; Support system: Establish a support network, including family members, friends, or members of the treatment team, to provide emotional and practical support to encourage patients to follow the treatment regimen; use of technological tools: apply cell phone apps or other digital tools to remind patients to take their medication on time or to participate in interventions.

3.6 Test endings

3.6.1 Main ending variables

Fugl-Meyer Assessment Upper Extremity Scale (FMA-UE) (6 weeks)

3.6.2 Secondary ending variables

(1) Sense of Ownership (SOO) Questionnaire

（2） Proprioceptive drift scale

（3） Action Research Arm Test (ARAT)

（4） National Institutes of Health Stroke Scale (NIHSS)

（5） Mini-Mental State Examination (MMSE)

（6） Surface electromyography (sEMG)

（7） Electroencephalogram (EEG)

（8） Functional magnetic resonance imaging (fMRI)

4. Statistical methods and sample size determination

4.1 Statistics and Analysis Program

Measurements of the three groups at three time points were statistically analyzed using SPSS25 software. All measurements were first tested for normality using the Shapiro-Wilk method. Measures that conformed to normal distribution were expressed as mean± standard deviation, and within-group comparisons were made using repeated measures ANOVA, whereby results at post-intervention and follow-up were compared to baseline (initial pre-treatment assessment). In case of statistically significant differences, further post-hoc multiple comparisons will be executed to observe specific differences between time points. Comparisons between groups will be made using one-way ANOVA and further two-way comparisons will also be performed if there are significant differences between groups. In addition, non-normally distributed measures were expressed as medians (upper and lower quartiles), and Friedman's test was used for within-group before-and-after comparisons, while Kruska-Walls H test was used for between-group comparisons. Differences were considered statistically significant at $P < 0.05$.

4.2 Sample size determination

This study was a randomized controlled study with FMA-UE at follow-up as the primary outcome indicator, so the sample size was calculated based on the change in FMA-UE scores at follow-up. Sample size was calculated using analysis of variance for comparisons between multiple group means, and the sample was calculated using the formula $n = {}/\varphi^2\left(\sum S_i^2/g\right)[\sum(\bar{x}_i - \bar{x})^2/(g-1)]$

The number of groups (g) was 3, and the number of participants in the interactive VR group, the immersive and interactive VR group and the control group was 1:1:1. According to previous studies, the mean value of each group was 35.4 35.7 46.5, and the standard deviation (σ) of the difference between each group was 15.3 18 14.2, and the mean standard deviation (σ) was 15.8 [26]. Requires two-sided test,α is the probability of type I error, set to 0.05; 1-β is the degree of certainty (test efficacy), set to 80%, so β is 0.2.K is the average distribution ratio of the sample size of each group of data, set to 1. According to α, β, v1, v2 look up the table to get the value. Using PASS15

software, the total sample size was calculated to be 96, and the sample size of each group was 32.Considering the 20% dropout rate, 120 patients needed to be included in the final study, and the sample size of each group was 40.

5. Adverse events

5.1 Adverse event monitoring

Adverse events in this study will be collected from the day of the intervention to the end of the participant's discharge from the group. Adverse events will be collected and recorded by the researcher at all times. The researcher will accompany the participant throughout the intervention period, while the participant may contact the researcher to report any adverse event that occurs during non-intervention time, and the researcher will determine the treatment measures to be given. In addition, the researcher will inquire about patients who do not report during the intervention according to the protocol requirements.

5.2 Definition of Adverse Events

5.2.1 Adverse events

Motion sickness: such as dizziness and nausea; eye strain and visual discomfort; prolonged wearing of VR headsets or related equipment may cause skin irritation, indentation or discomfort.

5.2.2 Serious Adverse Events

None

5.2.3 Unintended adverse events

None

5.2.4 Relationship between adverse events and test drugs/devices

Device malfunction or misuse, perceptual conflicts from immersive environments, physical discomfort from device wear, etc.

5.3 Clinical Research Risk Profiles

Inform patients in advance of possible side effects and promptly adjust the equipment or end the training when symptoms occur. (1) Shorten the training time or reduce the complexity of the virtual environment for patients who experience motion sickness and gradually adapt to it; (2) Arrange for patients to take regular breaks if they experience eye fatigue and visual discomfort, and adjust the brightness and contrast of the virtual scene appropriately; (3) Choose to wear a helmet that is lightweight, made of soft material and has good breathability to ensure that it does not compress the skin for a long period of time, and check and replace the liner regularly.

5.4 Start and end dates and time schedule of research projects

Starting date of the research project: 2024.11.01-2027.05.31

Time Scheduling:

2024.11.01-2025.04.30: Participant Intervention and Data Collection Phase 1;

2025.05.01-2026.05.31: Adverse reaction monitoring and adjustment, interim analysis;

2026.06.01-2026.11.30: Participant Intervention and Data Collection Phase II;

2027.12.01-2027.5.31: Data analysis and summarization of results, project reporting and presentation of results, paper writing and publication.

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Informed consent

Project name: A study of the effects and mechanisms of virtual reality features on upper limb motor function in stroke patients

Head: Prof. Yonghui Wang

Version No.: V1.0

Project date: October 25, 2024

Dear patient.

You are suffering from post-stroke upper limb motor dysfunction and we are inviting you to participate in a clinical research study. Participation in this study is entirely your choice. This informed consent form will provide you with information that you should read carefully and make a careful decision about whether or not to participate in this desired study. If you have any questions about this study, you may ask your doctor or the researcher for an explanation. You may discuss this with family and friends to help you decide whether or not to volunteer for this clinical study. You have the right to refuse to take part in this study or to leave the study at any time without penalty and without losing your rights.

If you agree to participate, we will need you to sign and date this informed consent form. You will be given a signed and dated copy to keep.

Your participation in this study is voluntary and this study has been reviewed by our Medical Ethics Committee.

[Research title] A study of the effects and mechanisms of virtual reality features on upper limb motor function in stroke patients

[Research Unit] Department of Rehabilitation Medicine (Rehabilitation Center), Qilu Hospital, Shandong University, China

[Principal Investigator] Wang Yonghui

[Research Sponsor] Self-financed

[Why is the study being conducted?]

Stroke is one of the leading causes of adult death and disability worldwide, with approximately 77.4% of survivors experiencing varying degrees of upper extremity motor dysfunction, increasing society's need for rehabilitation medicine. Although

advances in medical technology have reduced stroke mortality rates, the absolute number of stroke cases is rising as the population ages, pushing up social healthcare expenditures.

In recent years, emerging rehabilitation technologies based on neuroplasticity such as transcranial magnetic stimulation, transcranial direct current stimulation, rehabilitation robotics, and brain-computer interface technologies have received widespread attention. Notably, the emergence of virtual reality (VR) technology has also opened up new possibilities for rehabilitation therapy.

VR provides an immersive rehabilitation experience through its immersive, interactive, and imaginative properties (collectively referred to as the "3Is"), which facilitates neurological remodeling in stroke patients to improve motor and cognitive function. Many studies have been conducted to investigate the effectiveness of VR interventions in stroke rehabilitation, but most of them have focused on evaluating the effects of VR as a holistic intervention, while neglecting the important role that its intrinsic properties play in this process.

Studies have shown that VR plays an important role in post-stroke rehabilitation because it induces a body perception illusion, which is enhanced by the "3Is" of VR, making VR-based multichannel stimulation an effective means of remodeling the external representation of the brain. The Rubber Hand Illusion (RHI) experiment is a classic paradigm for the study of body illusions. In addition, many subsequent studies have tried different "virtual hand illusion" (VHI) experiments, which not only validate the findings of the classic RHI experiments, i.e., that a sense of possession can be induced in artificial or virtual hands, but also make the experiments more flexible. As VR technology continues to evolve, more new experimental paradigms for studying the sense of ownership or agency may emerge. However, the corresponding studies on the characteristics of VR have not been further generalized to practical clinical applications in post-stroke rehabilitation.

[How was this study conducted?]

Participants must be provided with the post-trial intervention before the study is conducted. This study was a single-center, single-blind randomized controlled study. An envelope drawing method was used to randomize the participants into three groups in a 1:1:1 ratio, A: interactive VR group, B: immersive and interactive VR group, and C: control group. Each group will receive the intervention four times a week for one hour for two weeks. The researcher will follow up with the participants

one month after the intervention. The primary outcome measure will be the Fugl-Meyer Assessment of the Upper Extremit (FMA-UE). The secondary outcome measures will include the "Sense of Ownership Questionnaire", "Proprioceptive Drift Scale", "Action Research Arm Test (ARAT)", "NIH Stroke Scale (NIHSS)", "Mini-Mental State Examination (MMSE)", "Surface Electromyography (sEMG)", "Electroencephalography (EEG)" and "Functional Magnetic Resonance Imaging (fMRI)".

Prior to the intervention, we conducted a VHI experiment on all experimental groups to help stroke patients reintegrate their cognition of the hemiplegic side of the upper limb. The specific experimental procedure is described below:

Participants were asked to sit at a table with their hands secured to the tabletop to ensure that they were comfortable and able to maintain this position for an extended period of time, and were given a commercially available HTC Vive head-mounted display (HMD, https://www.vive.com) to wear. The virtual scene presented in the HMD was a darkened room with a table of the same size and color as the real table in the experimental setting, and a realistic virtual hand model visible from the first-person perspective, which was positioned and posed to correspond to the participant's real hand. During the experiment, participants were required to keep their head stationary and their visual orientation constant. Previous studies have demonstrated that the sensitivity of the RHI is independent of hand laterality, so we tested only the affected hand.

We used a 6-degree-of-freedom handle to tap and stroke the participant's real hand, the position of which was tracked by the Intersense 900 system to determine the position of the virtual handle. Thus, when the experimenter moved the handle, the virtual handle was synchronized to reflect the movement. Participants felt the real handle tapping and stroking their hand while seeing the virtual handle doing the same to the virtual hand. This process lasted a total of 5 minutes.

To further enhance haptic feedback, we mounted a small stimulator on the back of the palm of the participant's affected hand to deliver vibrotactile stimuli to their hand. The stimulator was triggered by an Arduino microcontroller (https://www.arduino.cc) via a customized script developed in the Unity software environment (http://unity.com). During the experiment, a virtual ball appears in the virtual scene, which moves up and down according to a preset animation. When the virtual ball reaches the virtual part corresponding to the stimulator position, the

stimulator vibrates synchronously for about 500 ms. The stimulation phase lasted 60 seconds and was repeated six times.

A: Interactive VR group: similar to the VHI experiment, participants were required to wear an HMD and then enter a virtual experimental environment. The VR scenes in this group were based on basic motor interactions and contained a realistic model of the hemiplegic side virtual hand, a virtual sphere, and a virtual background identical to the real experimental environment, and they were all presented in a first-person view. The program commands the hemiplegic-side virtual hand to perform specific interactive tasks, such as grasping the virtual sphere - holding the virtual sphere for 3 seconds at a time for a duration of 3 seconds and then relaxing it for 3 seconds. At the same time, a real sphere of the same size and color as the virtual ball is also provided in the real world, and the participant is instructed to perform the same movement as synchronously as possible with the real hand on the hemiplegic side. The treatment was conducted four days a week for one hour per day for two weeks.

B: Immersive and Interactive VR Group: The virtual environments in this group were designed to combine rich immersive VR scenes with specific interactive tasks. Specifically, there were two virtual environments: (1) Natural scenery: participants were on a peaceful forest trail surrounded by tall trees, birdsong, and the sound of rushing water. In this scenario, the program commands the hemiplegic side virtual hand to grasp a spherical fruit on a tree, holding the fruit for 3 seconds at a time for a duration of 3 seconds, followed by 3 seconds of relaxation; (2) City park: the participant is in a city park surrounded by the sounds of children playing and the background sounds of the natural environment. In this scenario, the program causes the hemiplegic side virtual hand to grasp a ping-pong ball in the park, holding it for 3 seconds at a time and then relaxing it for 3 seconds. To enhance the sense of actual participation, a real sphere of the same size and color as the virtual object will be provided in the reality, and participants will be instructed to synchronize the execution of the corresponding movements with the real hand of the hemiplegic side as much as possible. The treatment will take place four days a week for one hour per day for two weeks.

C: Control group: the VR scene in this group does not have a complex storyline or visual effects, nor does it emphasize specific interactive tasks. It contains only a first-perspective realistic model of the hemiplegic side virtual hand and a virtual background identical to the real experimental environment. The program will have the

hemiplegic-side virtual hand perform simple and repetitive grasping motions, with each fist clench lasting 3 seconds, followed by 3 seconds of relaxation. Participants were also instructed to try to perform the same movements simultaneously with the real hand on the hemiplegic side. The treatment is performed 4 days a week for 1 hour per day for two weeks.

[Conditions for participation in the study]

This study plans to recruit 120 study participants.

1. Inclusion criteria: (1) First episode of stroke, hemorrhagic or ischemic, confirmed by computed tomography or magnetic resonance imaging of the brain; (2) Age 40-80 years, either male or female; (3) 3-12 months since stroke occurrence; (4) Mild-to-moderate or moderate-to-severe upper extremity impairment, with a FMA-UE score between 16 and 53; (5) Basic ability to communicate and comprehend the research instructions, with a score of 21 and above on the MMSE scale; (6) Agreement to participate in the study, with an informed consent form duly signed by the patient or a family member.

2. Exclusion criteria: (1) The presence of ferromagnetic metals implanted in the body; (2) Visual or hearing deficits; (3) Unstable medical conditions; (4) History of receiving similar VR training in the past; (5) A history of myasthenia gravis, multiple sclerosis, muscular dystrophy, or other diseases that may cause upper limb movement disorders..

3、Criteria for midway withdrawal were (1) the occurrence of other illnesses during the period that required specific therapeutic interventions, (2) voluntary withdrawal of informed consent, and (3) the occurrence of other unforeseen circumstances that precluded treatment or assessment.

[How long will my participation in this study last?]

Your participation in this study will last 6 weeks. During the first 2 weeks you will be treated in the Rehabilitation Medicine Department (Rehabilitation Center) and will be evaluated before, after and at the end of the 4th week of treatment.

[What are my responsibilities?]

If you decide to take part in this study, it is important that you come to your treatment on time, and come to the hospital at the follow-up appointments that your doctor has agreed to with you. Your follow-up visits are very important because your doctor will determine if the treatment you are receiving is really working and will be able to guide you in a timely manner. Please keep us informed of any changes in your

treatment.

[What will be done on each study visit?]

Changes in upper extremity motor function and the presence of discomfort during treatment.

[What are the possible risks of my participation in this study?]

The assessment and treatment involved in this study are all routine clinical treatments, in which the intervention method involved in this study is non-invasive virtual reality stimulation, which may cause motion sickness manifestations such as dizziness, nausea and other discomforts; eye fatigue and visual discomfort; and prolonged wearing of VR helmets or related equipment may lead to skin allergies, indentation or discomfort. (1) Shorten the training time or reduce the complexity of the virtual environment for participants who experience motion sickness to gradually adapt; (2) If eye fatigue and visual discomfort occurs can arrange for participants to take regular breaks, and appropriately adjust the brightness and contrast of the virtual scene; (3) Choose to wear lightweight helmets made of soft materials with good ventilation to ensure that they do not compress the skin for long periods of time, and check and replace the liners regularly.

[What are the possible benefits of participating in this study?]

Your upper extremity motor function may improve, and this study may help be used for other patients with similar conditions.

[What treatment options do I have if I don't participate in the study?]

Traditional treatments.

[What do I have to pay?

You will not have to pay for any of the treatments and evaluations that are part of the study protocol during the study. You will be responsible for the cost of treatments and tests if you have other medical conditions.

[Study on medical treatment and compensation for related injuries]

If any damage related to this study occurs, and the authoritative organization stipulated by national laws and regulations determines that it is necessary to bear the corresponding responsibility, the project team will provide you with free treatment and compensate you in accordance with national laws and regulations.

[What happens if I don't want to participate in this study or if I drop out of the study?]

You may choose not to participate in this study, or you may request to withdraw

from the study at any time by notifying the investigator that your data will not be included in the results of the study, and any of your medical treatment and rights will not be affected as a result.

[What will happen to my personal information?]

If you decide to take part in this study, your personal information about your participation in the study and in the study will be kept confidential. Your sample will be identified by the study number number and not by your name. Information that identifies you will not be disclosed to members outside the research team unless you give your permission. Your file will be accessible only to the researchers.

To ensure that the study is conducted in accordance with the regulations, members of the Ethical Review Committee will have access to your personal data at the research unit when necessary and as required. The results of this study will be published without disclosing any of your personal data.

[Who can I contact to learn more about this study?]

If you need further information about the study materials, or if at any time you feel that any of your symptoms are causing you problems, or if you are suffering from a study-related injury, please contact your study doctor/researcher.

[Who can I contact about my rights as a research participant?]

This informed consent and this study were approved by the Medical Ethics Committee (EC) of Qilu Hospital of Shandong University, a group of researchers and non-researchers who oversee research involving human participants. They follow the guidelines and rules of the State Food and Drug Administration (CFDA). If you have any questions about your rights as a research participant, please contact: Research Ethics Committee, Qilu Hospital, Shandong University (0531-82165409).

[Statement of consent]

I have read this informed consent form.

I had the opportunity to ask questions and all of them were answered.

I understand that participation in this study is voluntary.

I may choose not to participate in this study or withdraw at any time by notifying the researcher without discrimination or retaliation, and any of my medical treatment and rights will not be affected as a result.

The study physician may terminate my continued participation in this study if I need other treatment, or if I do not comply with the study plan, or if a study-related injury occurs or for any other reason.

I agree to participate in this clinical study and have received a signed copy of the Informed Consent Form.

Patient's (participant's) name (in block letters):

Contact phone number:

Signature of Patient (Participant):

Date

Name of patient's (participant's) legal representative (in block letters):

Signature of Patient's (Participant's) Legal Representative:

Date

Relationship with the patient (participant):

Contact number of the patient's (participant's) legal representative:

Signature of Patient's Guardian:

Date:

Relationships with patients:

Patient's guardian contact number:

Name of researcher (in block letters):

Signature of researcher:

Date

(Note: the signature of a witness is required if the participant is illiterate, or an agent if the participant is incapacitated).