

Research Article



Training Interventions Change Relative Power Spectrum of Alpha After Anterior Cruciate Ligament Deficiency in Athletes

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ABSTRACT

Introduction: In individuals with anterior cruciate ligament deficiency (ACLD), defective sensory and motor neuroplasticity occurs in the central nervous system (CNS) due to defects in sensory afferents. To successfully restore ACLD individuals to pre-injury conditions, it is necessary to modify the neuroplasticity created in the CNS by prescribing more appropriate training. For this aim, in this study, we used perturbation training differently.

Materials and Methods: Thirty athletes with unilateral anterior cruciate ligament (ACL) rupture were randomly assigned to the perturbation and standard training groups. The training program of two groups was performed in three intermittent sessions per week for one month. The relative power spectrum of alpha of quantitative electroencephalography (QEEG) was measured in three tasks: (1) the single-leg jump-landing, (2) the single-leg stance with opened eyes, and (3) the single-leg stance with closed eyes.

Results: The perturbation training group only showed significant symmetry in the relative power spectrum of alpha between the two limbs in the single-leg jump-landing test ($P=0.92$, $ES=0.04$) in comparison pre-post test. Also, this group showed high symmetry in the alpha band in the single-leg stance test with closed eyes ($P=0.53$, $ES=0.16$).

Conclusion: The results of the present study showed that both mechanical perturbation and standard training are suitable for transporting ACLDs back to sports. It also seems that mechanical perturbation training had higher effectiveness in modifying the CNS alpha power.

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1. Introduction

Following the rupture of the anterior cruciate ligament (ACL), the sensory organs that make up the mechanoreceptors of the ligament, including the free nerve endings, as well as the Ruffini, Pacini, and Golgi tendon organs, are damaged [1]. The extensive structural and functional changes in the sensory and motor neuroplasticity occurs in the cerebral cortex [1-5]. Due to defective neuroplasticity, functional instability of the joint [5, 6], a decrease in proprioceptive sensation [7-9], and balance disorder [10-12] occur. Despite rehabilitation and surgical treatments, there is a 25%-30% chance of re-rupturing the ACL in both knees [2, 5, 13, 14]. Approximately 50% of individuals experience early knee osteoarthritis and diminished health-related quality of life [2, 5].

It seems that in people with anterior cruciate ligament deficiencies (ACLs), compared to healthy people, more neurocognitive resources are needed to perform motor tasks [2]. Then, the activity in certain sensory areas of the brain increases, especially in the visual cortex, which indicates the need for increased visual data for compensatory mechanisms due to the lack of somatosensory afferents from the ligament to provide proper motor control and necessary joint stability [2, 3, 5, 14-16]. As a result, the ability of the nervous system to control feedforward ways to prepare for a quick and appropriate response to unexpected and sudden events and to deal with joint loads is reduced [2, 3, 5, 6, 14]. In addition, defective adaptation is formed in the cerebral cortex, which moves the movement strategies from a semi-automatic to a more voluntary state in the long term. They move their limbs more voluntarily than healthy people [4]. In the limited number of studies on ACLs before and after surgery, changes in the power of electroencephalography (EEG) frequency bands have been investigated to measure brain activity during sensory-motor tasks [2, 3, 5, 17, 18]. Unfortunately, the number of studies found on ACLs and assessing brain activity changes during the rehabilitation process is very low [2].

According to available evidence, subthalamic activity is mainly driven by cortical activity in both alpha (8-12 Hz) and beta (12-30 Hz) frequency bands, which are involved in attentional, executive, and motor planning functions [18-20]. Alpha frequencies are defined in two bands, alpha-1 (8-10 Hz) and alpha-2 (10-12 Hz), which play an important role in controlling balance [13, 19-21]. In ACLs, the processes related to attention, prediction, and execution, which are mainly related to alpha and beta frequencies, become defective and asymmetric. In

this study, changes in the amount and symmetry in the brain alpha power spectrum were investigated. Alpha band activity is associated with access to information that indicates the processing of environmental information [17]. It seems that alpha modulations respond to all cognitive domains (perception, attention, working memory, and long-term memory). Alpha band activity is determined as a decrease or increase in amplitude depending on the demand of the stimulus or task [20]. Alpha band activity and attention behavior and its role in the active inhibition of the cortical network are agreed [20, 22]. Usually, when the cognitive load is high, such as when the eyes open, the alpha activity decreases. This decrease indicates the stimulation or activation of the cortex. Inversely, if the alpha activity increases, it inhibits the brain. The high alpha activity is recorded in conditions where there is no need for attention or if the eyes are closed and the semantic load is low. The brain regions that are activated during a task show lower alpha activity, and areas unrelated to the task and interfering processes show higher alpha activity. Increased alpha activity creates more precise timing and inhibits additional information processing [20]. Therefore, the power of the alpha band increases in brain areas unrelated to the task, in activities that require attention and cognition, and irrelevant information should be removed [20, 23]. Miao et al. (2017) showed that the power of all frequency bands, including alpha, was significantly higher in the ACLs than in healthy subjects while performing functional tasks. Probably these individuals, due to the defects in afferent information, inhibit unnecessary visual processing in brain areas by increasing alpha band activity to reduce the entry of information unrelated to the task. Also, the power of EEG signals in the ACLD group compared to the healthy group was asymmetric in the two cerebral hemispheres [18].

According to proprioceptive studies [7-9] and brain activity studies [1, 6, 18] in ACLD individuals, asymmetry in cortical activity and motor control of limb movements was demonstrated. One of the main goals in the treatment of individuals with ACLD is to eliminate the asymmetry in motor control in performing daily and sports activities [24, 25]. Therefore, rehabilitation exercise programs should be designed in such a way that they have long-term effects on the memory and transfer of skills and be sufficiently challenging and motivating for the individual [4, 24, 25].

Management of the return to sports in ACLDs includes both conservative and surgical treatments [2, 3, 6]. Ligament reconstruction surgical treatments cannot correct the sensory function of the grafted ligament, and its suc-

cess is only in removing the mechanical defect. After the surgery, motor defects remain and the sensory connection of the ligament with the central nervous system (CNS) is not corrected [2, 3, 26]. Therefore, individuals always show increased brain activity in sensory and attention areas [3, 15, 27]. More recent studies have shown that the basis of the treatment in both rehabilitation and surgery should be training that can strengthen automatic and unconscious processes. There is an emphasis on training that focuses on external attention and activities that require anticipation because external focus instructions and dual tasks increase intracortical inhibition, which was reduced following ligament rupture. In this way, more strengthening and coordination between the muscles, especially the quadriceps, in motor control is achieved [10, 24, 25, 28-30]. Very few studies have been conducted on treatments that can change cortical function during sensory-motor tasks, including rehabilitation techniques in the field of strength, balance, and perturbation training [2, 6]. Researchers believe that visual-sensory control factors should be considered especially in the final stages of rehabilitation of these individuals [14, 24, 25]. As the CNS is the executive control center of the entire proprioceptive sensory pathway and plays an essential role in the functional stability of the knee joint [18], most of the motor deficits in the lower limbs are probably related to the asymmetries and the change in the power of the frequency bands of the cortex. There are imperfections in the current therapeutic exercise programs for ACLD individuals in acknowledging the existence of disorders in the bottom-up sensory system and the top-down control system of the brain. Despite that, there are some therapeutic approaches toward treatments based on the principle of feedforward and focusing external attention in the form of designing perturbation training [4, 5, 25, 31, 32]. During the initial stages of motor relearning in ACLDs, movement execution requires more attention, which results in conscious control of movement, and this motor learning model contrasts with automatic motor control processes that normally regulate movements [3, 28]. Evidence shows that training that focuses on internal attention or visual and auditory feedback increases cortical activity and reduces the ability to maintain motor patterns, but training that emphasizes external attention and reduces feedback can help to activate the automatic areas of the subcortical brain [3, 6]. Studies conducted in the field of training and motor learning showed that exercise changes the power of the alpha and beta bands. It is possibly due to functional reorganization related to motor learning in the brain [33]. Motor learning is associated with progressive increases in alpha band power and reduced attentional

demands [21, 33, 34]. In the present study, a comparative study of the EEG alpha power spectrum in two types of training was performed: (1) mechanical perturbation training in all planes and axes of movement with the gradual addition of cognitive loads during therapy sessions and (2) standard strength-endurance training of sport rehabilitation program. We then used challenging functional tests for measurements; (1) single-leg jump-landing and maintaining balance, (2) single-leg stand with eyes open, and, (3) single-leg stand with eyes closed in coper ACLDs. In this study, the trend of EEG alpha power changes in healthy and injured limbs in each group was investigated and also a comparison of the two groups was done. We hypothesized that training can modulate neuroplasticity due to the process of learning and reorganizing the CNS, as well as reducing brain activity in controlling balance movements. We expected that in both groups, in performing the three tested tasks, the relative power spectrum of the alpha of two limbs would be more symmetry and increase. We also expected that in the between-group comparison, the perturbation training group would show more improvement than the standard training group. The results of the study can be used to define a faster and non-invasive treatment to return athletes to sports and competitive environments.

2. Material and Methods

Study participation

Thirty people (17 men and 13 women in the age range of 18 to 40 years) with complete unilateral rupture of the anterior cruciate ligament of the left knee were selected from the athletes who were referred to the clinic of the Sports Medicine Federation of the Islamic Republic of Iran (SMFIRI). All participants were first diagnosed by an orthopedic surgeon through clinical tests and magnetic resonance imaging (MRI) images and then referred to a sports physiotherapist to evaluate the inclusion and exclusion criteria. The inclusion criteria were being an athlete (that is, someone who exercises approximately 10 hours a week and whose resting heart rate is approximately 60 beats per minute or less [35]), having at least two months have passed since their injury and they do not intend to undergo surgery for at least six months, having received at least ten sessions of physical therapy to have a full range of motion, absence of pain and swelling, sufficient strength of the quadriceps muscles, and being able to perform hop on the affected limb without pain, discomfort, or fear. The exclusion criteria were injury in other joints of the lower limbs and spine in the last six months, surgery in these areas in the last year, having pain and swelling and limited movement in the

knee and other joints of the lower limbs, the medical prohibition of performing activities by the orthopedic surgeon for any reason, suffering from a systemic disease, such as diabetes or any other disease that requires the routine use of a specific effective drug, any history of weakness, muscle atrophy, numbness and tingling of the limbs, and back pain in the neuromuscular system, a history of an ACL tear on the opposite side, nervous disorders, mental problems, dizziness, fear, anxiety, and stress from entering the study and not having mental health, and withdrawing from the plan by the participant for any reason and at any time. Then, they were divided randomly using a simple allocation to the perturbation training group and also to the standard training group. Candidates who were suitable to participate in this study were copers as explained in the protocols of previous studies [5, 32, 36, 37]. This study was approved by the

Ethics Committee of the Sports Sciences Research Institute (SSRI) (Approval ID: IR.SSRC.REC.1399.095) and all participants accepted and signed the consent form to participate in this study before entering the study.

Training interventions

Standard training group

The training protocol of this group was cardiovascular, lower extremity muscle strength, balance, core stabilization, agility, and sport-specific exercises (Table 1). The training program of this group of participants was performed for three intermittent sessions a week for a month.

Table 1. Guidelines for progression of training in the standard training group

Type of Training	Activities	Timing	Difficulty Progression
Cardiovascular	<ul style="list-style-type: none"> - Stationary bike - Stepper - Outdoor running 	<ul style="list-style-type: none"> - 10 minutes - 5 minutes - 30 minutes 	<ul style="list-style-type: none"> - 60 to 80 rpm - Up to 10 minutes - Flat ground to uphill
Lower extremity muscle strength	<ul style="list-style-type: none"> - NMES - Standing squat (0 to 80 degrees) - Sitting (90 to 35 degrees of knee extension) - SLR (0 to 45 degrees of hip flexion) - Weight machines - Extension & flexion of leg curls - Leg press - Elastic bands - Hip movements in four directions - Terminal knee extension - Lunges - Forward - Side 	<ul style="list-style-type: none"> - 10 minutes (10 seconds contraction, 15 seconds rest) - 15 RM, 5 sets, 10 reps - 3 sets, 10 reps - 3 sets, 10 reps 	<ul style="list-style-type: none"> - Without weight cuffs - Two legs to one leg - Low to increase strength - Without elastic bands
Balance	<ul style="list-style-type: none"> - Circular balance board - Straight knee - Semi-squat knee 	<ul style="list-style-type: none"> - 3 sets, 30 seconds 	<ul style="list-style-type: none"> - Open eyes to closed eyes
Core stability	<ul style="list-style-type: none"> - Plank - Crunch - Side planks - Leg scissors crunch - Single-leg bridge 	<ul style="list-style-type: none"> - 3 sets, keep within tolerance for seconds - 3 sets, 10 reps 	<ul style="list-style-type: none"> - Increasing holding time
Agility	<ul style="list-style-type: none"> - Running fast in all directions with sudden starts and stops - 8-figure running - Side sliding to the right and left with sudden stops - Fast forward and backward shuttle run with sudden starts and stops - 45 degrees cutting spinning drill 	<ul style="list-style-type: none"> - Tolerate speed training without pain or apprehension 	<ul style="list-style-type: none"> - To full speed
Sport-specific	<ul style="list-style-type: none"> - Routine sport form 	<ul style="list-style-type: none"> - Tolerate practice without pain or apprehension 	<ul style="list-style-type: none"> - Gradually to full sports-specific skills



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Figure 1. The figures show the general panel of the perturbation device with two protective handles mounted on the wall for the participant to hold if they feel unbalanced. The right image shows a participant standing on the perturbation plate while flexing his knees to achieve more stability, and the left one shows a standby participant standing with extended knees on the plate. For more information about the characteristics of the mechanical perturbation device and the training method, refer to the authors' previous articles [5, 32].

Perturbation training group

In the perturbation group, a perturbation plate was used that created movements on all motion plates (angular, horizontal, and vertical), and the simultaneous combination of all horizontal, vertical, and angular modes was used (Figure 1).

This device was used in two ways: as a means of dynamic warming up of the muscles of the participants in the closed chain of motion in the front-right and front-left angular movement axes (as an internal-perturbation training), and as a stimulus to induce unpredictable pos-

tural neuromuscular reactions in participants (external-perturbation training or main training). In participants who were randomly assigned to the perturbation group, exercise therapy was performed alternately for one month and three sessions per week as follows (Table 2):

1. Warm-up stage (internal-perturbation training): In this stage, the participant first rode a stationary bicycle for five minutes, then he/she stood in the functional position on the pre-prepared exc-balance and tried to apply push on the device in the adjustment directions (in two angles: front-right and front-left), and did this for

Table 2. Guidelines for progression of training in the perturbation group

Type of Training	Activities	Timing	Difficulty Progression
Warm-up (Internal-perturbation)	<ul style="list-style-type: none"> - Stationary bike - Internal perturbation - Front-right - Front-left 	<ul style="list-style-type: none"> - 5 minutes - 3 sets, 15 repetitions alternately for each side, maximum speed & power 	-
Main exercise (external perturbation)	<ul style="list-style-type: none"> - Therapist randomly & unpredictably disturbs participant balance in different directions: <ul style="list-style-type: none"> - Front-right angle - Front-left angle - Rear-down angle - Antero-posterior horizontal - Medio-lateral horizontal - Up-down - The combination of the six above modes 	<ul style="list-style-type: none"> - Suddenly & randomly, every 30 attempts, 1 minute's rest, low speed of change perturbation, focus with a look at the plate (Internal attention), keep handles, eyes open, training without pain or apprehension 	<ul style="list-style-type: none"> - Increase the speed of perturbation - Look forward and straight the head (external attention) - Release handles - Close eyes
Cool-down	<ul style="list-style-type: none"> - Static stretching movements - Hamstrings - Quadriceps - Triceps surae - Stationary bike - Ice pack for knees 	<ul style="list-style-type: none"> - 3 sets, 15 seconds for every part - 5 minutes - 15 minutes 	-

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15 repetitions in each direction at the maximum speed and power he/she could create in his/her muscles. This internal-perturbation exercise was repeated immediately three times in both directions. Therefore, that participant first did 15 repetitions with maximum speed on the right and immediately did 15 repetitions with maximum speed on the left and did the same thing alternately for the next two repetitions. The time interval between the exercises for each side was considered as the rest time for the other side. Then, the participant descended from the device and rested for one minute, during which the physiotherapist adjusted the device to perform an external-perturbation exercise.

2. Main exercise stage (external-perturbation training): In this stage, the participant stood on the exe-Balance device, and the physiotherapist randomly unpredictably disturbed the participant's balance, who was trying to maintain his/her postural stability in different directions of the device and the participant's attempt to maintain his/her postural stability was suddenly disturbed. A total of 90 training attempts were considered for each participant in this stage, with a break for one minute between all 30 exercises.

3. Cool-down stage: After performing the internal-external perturbation exercises, all participants performed cool down for 10 minutes.

Testing procedure

The study method was performed between 9:00 AM and 13:00 PM. The participants were asked to sleep at 9:00 PM, avoid eating caffeinated substances on the day of the test, and they had not eaten anything for one hour before the test. An objective examination of the participants was performed in the form of brain electrical activity tests in 21 registered active electrodes in two forms: pre-test (one to two days before entering the intervention training program) and post-test (one week after the end of the training period of intervention) in three challenging functional tests in the form of single-leg jumping-landing on one leg and maintaining balance after landing, single-leg balance with eyes open, and single-leg balance with eyes closed. To perform the single-leg jump test, 50% of each participant's height was determined and marked on the ground [38-40]. Then, the subject is stranded on a marked line and, at the command of the examiner, raises the non-test leg and jumps forward (about 50% of the height or more). The examiner recorded each jump and landing test five seconds before the announcement of the verbal command and 5 seconds after finishing the jump process so as not to delete any data. The

recording moment was determined by recording the trigger on the EEG software. The EEG recording characteristics of each participant's jump and landing were saved in three series of repetitions to be used in the analyses. The tests were repeated exactly for the opposite limb.

Balance tests were performed standing on one leg, once with eyes open and once with eyes closed for both limbs. To perform standing tests on one leg, first, each participant stood on both legs and while looking at a point in front and at eye level, which was located two meters away, with the command of the examiner, the participant raised the non-test leg and tried to maintain this position for 30 seconds while trying to avoid body sway as much as possible. At the end of the test, the examiner informed the participant that the test was over and the participant was allowed to rest for one minute. The next test was repeated for the opposite side afterward. The eyes-closed single-legged standing test was performed exactly like the eyes-open single-legged standing test, except that when the participant raised the non-test leg, the eyes were closed while the head was raised while trying to keep the body balanced. The examiner recorded each balance test five seconds before the announcement of the verbal command so as not to delete any data. The recording moment was determined by recording the trigger on the EEG software. The EEG characteristics of each participant's single-leg standing with open and closed eyes were saved in three series of repetitions to be used in the analyses. The tests were repeated for the opposite limb as well.

EEG data collection and spectral analysis

In this study, a Liv intelligent technology 32-channel wireless electroencephalogram device with input impedance specifications of 10M, bandwidth of 2 kHz, and 24-bit image resolution made in Iran, was used. After placing the device cap on each participant's head, the recording areas were cleaned and rubbed with alcohol. Then, by using a blunt needle, we inserted the gel into the series of electrodes contact with the scalp, then in the laptop connected to the EEG device wirelessly, we checked each electrode so that its impedance reached less than 10 k Ω and the corresponding light in the software was turned on. After all the recording electrodes were turned on, the subject was ready to be tested. In this study, 21 areas of the 32 brain regions, including Fp1, Fp2, F7, F3, Fz, F4, F8, T7, C3, Cz, C4, T8, P7, P3, Pz, P4, P8, O1, O2, Fpz, and Oz were recorded for further analysis.

The raw data were recorded by the eLORETA software at first de-noised and then used for calculating the rela-

tive power spectrum of the brain waves for three tasks in all 21 electrodes in the form of quantitative analysis of brain waves (QEEG) in the pre- and post-tests for all participants in both right and left limbs for the whole brain. The EEG analyses in this study were exclusively done on the alpha frequency band.

For the spectral analysis of alpha, we calculated power spectral densities (PSD) using Welch’s method, or “pwelch” function in MATLAB (ver. 2019a, Math-Works Inc., Natick, MA., USA). For PSD estimation, we chose a 2-s Hann window with a 50% overlap between segments. This resulted in a frequency resolution of 0.5 Hz to capture the changes in spectral activities in our EEG data. We then transformed PSDs into relative powers to allow for comparisons. To calculate relative power in μV^2 , we divided PSD by the sum PSD from all bins for each frequency bin within 0 and 40 Hz and each electrode. Alpha band powers from the whole of the cortex were calculated for each test by averaging activities from 21 electrodes and summing the relative powers of all bins within 8-12 Hz.

Statistical analysis

In order to investigate the effect of the type of therapeutic intervention (perturbation training or standard training) on the EEG alpha power spectrum in single-leg jump-landing and standing on one leg with open and closed eyes tasks in the pre- and post-tests for intragroup and intergroup comparisons we used multivariate analysis of variance (MANOVA). Then, one-way ANOVA and supplementary tests were used to compare the effects of therapeutic interventions on intergroup comparisons in the post-test and intragroup comparisons, and also to compare the differences between two limbs for the cortical alpha power spectrum variable. Afterward,

the $P=0.05$ was used as a significance level to compare the effect of the type of therapeutic intervention between the two groups. We used the effect size of Cohen’s d test (ES) to statistically determine how much the type of treatment affected the investigated variable. Cohen’s d is an appropriate effect size for the comparison between two means. Because in the present study, in the comparisons made between two limbs in each group, we were looking for symmetry (means, the P at the level of non-significance), we interpreted the results based on this explanation: the closer the ES value is to zero (that is, the should be closer to one) that is, the differences in the comparison between the two limbs and between the two groups are less, and the two limbs are closer to symmetry, and there is no difference between the two types of treatment methods. Also, the closer the ES value is to one (which means, the P is closer to the significance level), the two limbs and two groups are different and asymmetric in terms of comparison. For interpretation of ES, Cohen suggested that $0.15 > d \geq 0.15$ can be considered a ‘negligible’ effect size, $0.4 > d \geq 0.15$ represents a ‘small’ effect size, $0.75 > d \geq 0.4$ represents a ‘medium’ effect size, $1.1 > d \geq 0.75$ represents a ‘large’ effect size, $1.45 > d \geq 1.1$ represents a ‘very large’ effect size, and $d > 1.45$ represents a ‘huge’ effect size. This means that if the difference between two limbs or two groups’ means is less than 0.15 standard deviations, the difference is negligible, even if it is statistically significant [12]. For statistical analysis, we used IBM SPSS software, version 22.

3. Results

The characteristics of the participants in terms of age, weight, height, body mass index, pain level, and gender are given in Table 3.

Table 3. Characteristics of participants in groups

Variables	Perturbation		Standard	
	Mean±SD	Range	Mean±SD	Range
Age (y)	22.93±5.15	24-39	23.6±2.32	21-29
Weight (kg)	69.3±7.51	57-83	76.75±7.03	62-90
Height (cm)	172.0±3.25	168-178	180.0±7.3	170-194
Body mass index (kg/m ²)	23.38±2.27	26.4-19	23.45±1.65	20.2-26
Pain scale	5.6±1.5	2-7	3.13±1.35	0-5
Gender	6 females, 9 males		7 females, 8 males	

Table 4. Comparison of QEEG variables in the single-leg jump-landing test

Comparison of Groups			Relative Power Spectrum of Alpha (μV^2)	
			nAL*	AL**
Perturbation group	Pre-test	Mean \pm SD	21.69 \pm 8.36	25.82 \pm 11.62
	Post-test	Mean \pm SD	20.98 \pm 7.96	20.68 \pm 8.47
	P interlimb (post-test), ES		0.92, 0.04	
Standard group	Pre-test	Mean \pm SD	17.39 \pm 6.02	25.21 \pm 8.25
	Post-test	Mean \pm SD	19.2 \pm 6.8	25.39 \pm 7.84
	P interlimb (post-test), ES		0.07, 0.86	
Intergroup differences	P	Pre-test	0.12	0.87
		Post-test, ES	0.62, 0.24	0.13, 0.6
Pillai's trace			0.27	

*nAL: Non-affected limb; **AL: Affected limb.

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QEEG analysis of single-leg jump-landing test

The relative power of the alpha wave after the training period in the perturbation training group compared to the standard training group showed a medium decrease in ACLD limb jumping (P=0.13, ES=0.6) while showing a small increase in healthy limb jumping (P=0.62, ES=0.24) (Table 4). In the interlimb comparison, the relative power of the alpha in the perturbation training group showed a decrease in the post-test of both limbs, which was not observed in the control group. Symme-

try in the comparison of jump-landing on the ACLD and healthy limbs with negligible effect was excellent in the perturbation training group (P=0.92, ES=0.04), but with large effect was very low in the standard training group (P=0.07, ES=0.86). This means that the participants in the perturbation training group achieved a higher symmetry in the jump-landing task on both limbs compared to the standard training group (Figure 2).

Table 5. Comparison of QEEG variables in single-leg stance with opened eyes

Comparison of Groups			Relative Power Spectrum of Alpha (μV^2)	
			nAL*	AL**
Perturbation group	Pre-test	Mean \pm SD	20.7 \pm 10.17	23.62 \pm 9.7
	Post-test	Mean \pm SD	16.02 \pm 7.8	22.24 \pm 13.78
	P interlimb (post-test), ES		0.21, 0.58	
Standard group	Pre-test	Mean \pm SD	19.94 \pm 7.38	24.24 \pm 9.85
	Post-test	Mean \pm SD	16.08 \pm 6.84	20.2 \pm 3.7
	P interlimb (post-test), ES		0.07, 0.78	
Intergroup differences	P	Pre-test	0.82	0.84
		Post-test, ES	0.62, 0.01	0.58, 0.21
Pillai's trace			0.97	

*nAL: Non-affected limb; **AL: Affected limb.

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Table 6. Comparison of QEEG variables in single-leg stance with closed eyes

Comparison of Groups			Relative Power Spectrum of Alpha (μV^2)	
			nAL*	AL**
Perturbation group	Pre-test	Mean \pm SD	18.34 \pm 7	20.2 \pm 4.37
	Post-test	Mean \pm SD	18.6 \pm 10.4	19.87 \pm 5.8
	P interlimb (post-test), ES		0.53, 0.16	
Standard group	Pre-test	Mean \pm SD	21.85 \pm 7.6	23.26 \pm 7.22
	Post-test	Mean \pm SD	14.18 \pm 6.21	23.62 \pm 9.85
	P interlimb (post-test), ES		0.005*, 1.19	
Intergroup differences	P	Pre-test	0.2	0.17
		Post-test, ES	0.17, 0.53	0.21, 0.48
Pillai's trace			0.1	

*nAL: Non-affected limb; **AL: Affected limb.

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QEEG analysis of single-leg stance with opened eyes

In the standing test with eyes open on ACLD and healthy limbs, no significant difference was found between the two training groups (Table 5). In the comparison between two limbs only in the healthy limb of the perturbation training group, there was a medium decrease ($P=0.03$, $ES=0.53$), and in other values, the differences were not noticeable. However, the relative power spectrum of alpha in ACLD and healthy limbs of both groups decreased. The perturbation training group showed a medium effect ($P=0.21$, $ES=0.58$) and the standard training group showed a large effect ($P=0.07$, $ES=0.78$) in the symmetry of two limbs in the post-test (Figure 3).

QEEG analysis of single-leg stance with closed eyes

The relative power of the alpha band of the perturbation training group did not change for both limbs, and the standard training group showed a significant decrease only in the healthy limb (Table 6). In the comparison of the symmetry of two limbs in the post-test, the perturbation training group with a small effect showed very good symmetry ($P=0.53$, $ES=0.16$), and the standard training group with a very large effect did not show similarity between two limbs and mostly showed asymmetry ($P=0.005$, $ES=1.19$) (Figure 4).

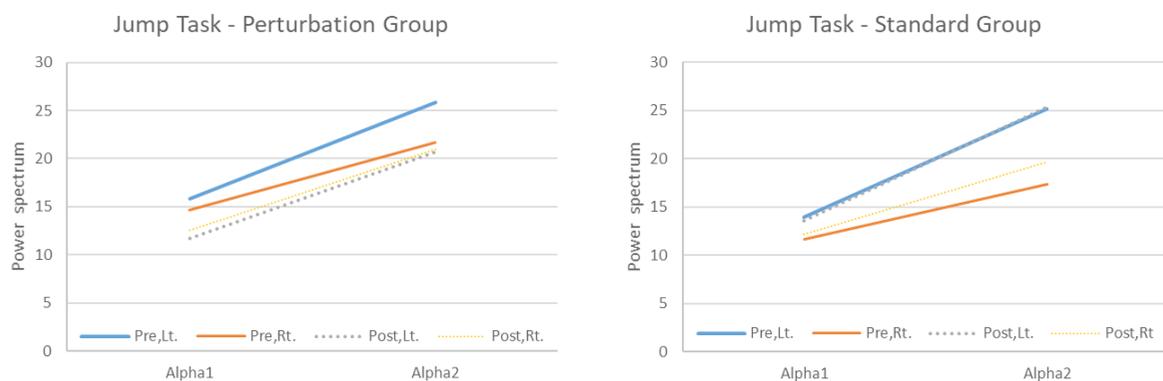
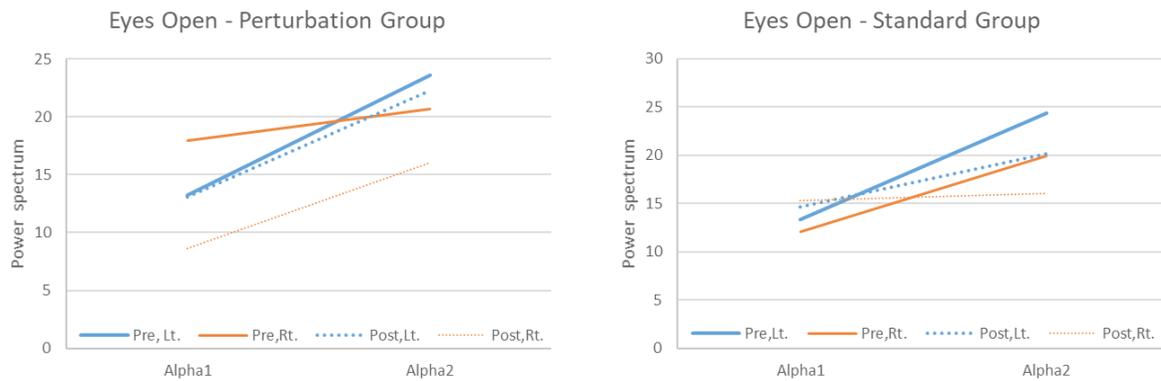


Figure 2. There was no significant difference in the relative power spectrum of alpha between groups in the single-leg jumping task on the ACLD (left) and non-affected (right) limb. But in the interlimb post-test comparison, the perturbation training group with negligible effect between two limbs achieved greater symmetry than the standard training group. *Alpha1 (8-10 Hz) and Alpha2 (10-12 Hz).

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Figure 3. In the single-leg standing test with eyes open, the relative power spectrum of alpha in both limbs and in both groups showed a declining trend. In the interlimb comparison, the perturbation training group showed low symmetry and the standard training group showed moderate symmetry of the alpha power spectrum between two limbs. *Alpha1 (8-10 Hz) and Alpha2 (10-12 Hz).

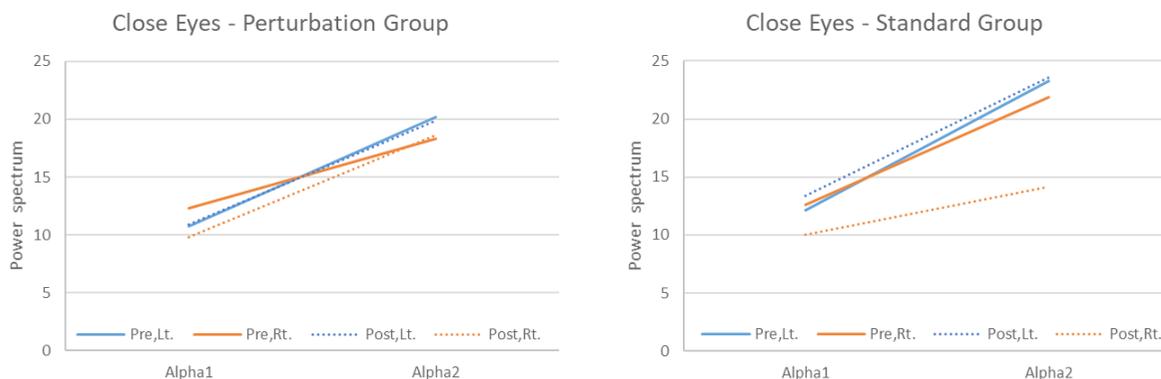
4. Discussion

In this study, we used EEG to determine the changes in the relative power spectrum of alpha in copers’ ACLDs in two groups following one month of mechanical perturbation training and standard training in three tasks of single-leg jump-landing, and single-leg stand with eyes open and closed. To increase the balance control demands, the tests, which all were performed on one leg with eyes open and closed were used. According to our hypothesis, both training groups almost achieved a higher symmetry in the relative alpha power in the post-tests but it was more in the perturbation training group. Also, we expected that by making the task more difficult, for example, jumping on one leg and maintaining balance after landing, as well as standing on one leg with eyes closed, the relative power of the alpha band would increase, but contrary to our hypothesis, the results of

the tests showed a decrease in alpha power or no change, which this decrease in alpha band power was probably due to the high challenge of balance tasks.

The necessity of using perturbation training versus standard training in ACLDs

Considering that the most effective treatment algorithm agreed upon for ACLDs athletes is “mechanical perturbation enhanced rehabilitation” [5, 25, 32, 37, 41-46], our main goal in this study was to optimize this type of treatment. In this study, to investigate the activity of the cerebral cortex before and after training, a mechanical perturbation device was designed and built by creating a perturbation in all movement axes to simulate similar events in the real world in more realistic acceleration and speeds. In comparing the present study with previous studies in the field of perturbation interventions, it can



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Figure 4. The perturbation training group did not change in the comparison of the pre- and post-tests and showed very good symmetry between two limbs in the post-test. The standard training group showed a considerable decrease only in the healthy limb, and in the post-test comparison, we found more asymmetry in alpha power spectrum between the two limbs. *Alpha1 (8-10 Hz) and Alpha2 (10-12 Hz).

be said that none of the devices that created mechanical perturbation movements included all movement axes and were often limited to two movement axes [37, 43, 44]. In a review of the available literature, no study was found on the effect of a period of perturbation training on brain activity in ACLD or healthy individuals. In the past, it was thought that balance is controlled by sub-cortical structures, but more recent evidence emphasizes that the cerebral cortex is definitely active in controlling compensatory reactions in unpredictable balance perturbations to maintain postural stability to prevent falls [13, 47]. Our hypothesis was that both groups would show more symmetry in the relative power spectrum of alpha after a one-month training intervention in the comparison between the two limbs. We also expected symmetry to be greater in the mechanical perturbation training group. The results of our study showed excellent symmetry in the task of jumping on the right and left leg and maintaining balance on the same limb in the post-test of the perturbation training group with an average decrease in alpha power in jumping on the left limb compared to the standard training group. In the task of standing on one leg with eyes open, the perturbation and standard training groups showed a non-significant decrease in both limbs, which in comparison, both groups showed moderate symmetry between the two limbs. In the task of standing on one leg with eyes closed, relatively good symmetry was obtained in the perturbation training group, while in the standard training group, the symmetry between the limbs was very poor. According to the existing studies in the field of EEG alpha activity, it can be concluded that the suppression of alpha activity recorded in the perturbation training group following the jumping task and the one leg standing task with eyes open, reflects the greater activation of the cerebral cortex. Usually, the more meaningful information retrieved from long-term memory, the greater the suppression of alpha activity occurs [20]. Also, when attention is directed to external visual events, alpha power decreases with increases in the visual cortex, this decrease in alpha power with external attention occurs due to the increased excitability of the sensory cortex to increase stimulus processing [48]. The results of our study, in line with the mentioned studies, mainly showed a decrease in the relative power of the alpha. The results of these studies were different from studies that examined the alpha power spectrum after training in different conditions from our study [33, 34]. In other studies, researchers showed that as the balance task becomes more difficult, for example, performing balance tasks with eyes closed or maintaining balance while standing on one leg when the movement becomes more challenging and the need for balance

control increases, a decrease in can be seen alpha power [13, 49, 50]. These results confirmed the results of our study. However, electromyographic studies have shown that both feedback and feedforward controls are enhanced by using perturbation training, unlike other types of training [24, 25, 31]. Also, unpredictable perturbation training affects the somatosensory afferents, modulates the defective function of the gamma loop, and as a result, knee dynamic stability is obtained in this type of training compared to other types [3, 32, 37, 41-44, 46]. In our study, after a period of perturbation training, brain alpha activity symmetry was obtained in the two limbs test, especially in the single-leg jump and single-leg stand with eyes closed test. It is suggested that perturbation training on mechanical plates by changing brain neuroplasticity and creating balanced control mechanisms from top to down while performing functional activities can be effective in transferring to other more complex motor activities in ACLD individuals.

The effect of type of training on the relative power spectrum of alpha in the single-leg jump-landing task

Our hypothesis was that different types of training can modulate neuroplasticity due to the process of learning and reorganizing the CNS, as well as reducing brain power in controlling balance movements. We expected that in both groups, in performing the three tested tasks, the relative power spectrum of the alpha of two limbs would be more similar and increase. We also expected that in the intergroup comparison, the perturbation training group would show more improvement than the standard training group.

In a study conducted by Miao et al. (2017), in order to investigate the changes in the characteristics of the power spectrum of EEG waves on ACLD and healthy people in the landing task, it was shown that the power of all frequency bands, including the alpha band, was significantly higher than the healthy group. Also, these researchers reported that the power of EEG signals in ACLDs was asymmetric in the two hemispheres [18]. The results of our study on the alpha power spectrum in the pre-test of the single-leg jump-landing task in both perturbation and standard training groups in the comparison of right and left limbs were asymmetric, which was confirmed by Miao et al. Because we did not have a healthy group for comparison, we cannot comment in particular on the level of power changes of frequency bands. On the other hand, in the post-test of the jump-landing task on each limb, we examined the relative power of the alpha spectrum in the whole brain, in which

the perturbation training group had excellent symmetry and the standard training group showed very little symmetry in comparison. It seems that the symmetry obtained in the power of the alpha spectrum of the cerebral cortex in the perturbation training group emphasizes the greater effectiveness of perturbation training compared to standard training in more challenging balance tasks. Studies have shown that performing long-term exercises strengthens brain mechanisms that can integrate visual, vestibular, and somatosensory inputs for balance [49]. Motor learning occurs with practice and it causes functional reorganization in the brain that is associated with amplitude changes in alpha (8-12 Hz) and beta (13-30 Hz) frequency [33]. The process of motor learning is associated with a gradual increase in alpha power, and this alpha modulation is related to the reduction in attentional demands likely to occur after skill acquisition [33, 34]. In this study, we expected to have an increase in alpha power in the post-tests following the one-month training period in both training groups, but the results in the single-leg jump-landing test showed a decrease in the power of the alpha band, which is contrary to the studies on healthy people. Probably, this difference in our study is due to the different conditions of the test and different subjects because the single-leg jump-landing test in our study, unlike the tests of the above studies, was performed in highly challenging balance conditions and also on deafferented ACLD individuals. The existing balance studies were not a suitable comparison criterion for our study. No study was found on the relative power of brain activity in the same training with the same test on ACLD individuals.

In the post-test of the single-leg jump-landing task of the perturbation training group, after the training, a great symmetrical reduction in the power spectrum of the cerebral alpha spectrum was obtained in jumping on each limb. This decrease in alpha power is related to the greater challenge of maintaining balance and external sensory attention. EEG studies in balance tasks have shown that alpha power in activities that require more external sensory attention is associated with a decrease in brain parietal alpha power, which means an increase in spatial vestibular sensory attention that is associated with increased balance demands [20, 48, 50]. Physiologically, it is thought that the decrease in alpha power due to external visual events is due to the increase in excitability of the sensory cortex to increase stimulus processing [48]. Based on the mentioned studies, in the single-leg jump-landing task, where there are both high balance challenges and a lot of external attention, it is expected that the decreasing activity of the alpha spectrum is normal, and we suggested that the researchers have

a healthy comparison group in subsequent studies. On the other hand, the symmetry observed in the reduced alpha power could be a reason for the more beneficial effect of perturbation training over standard training. Also, it seems that the power of the alpha spectrum in the single-leg jump-landing task is not a suitable criterion for evaluating the effectiveness of the type of training, and only by referring to the more symmetrical power of the spectrum in this task, the effectiveness of the type of training can be cited.

The effect of the type of training on the relative power spectrum of alpha in maintaining balance in tasks of single-leg standing with eyes open and closed

In the review of studies, no research was found on the effect of the type of training on single-legged balance with opened and closed eyes. Studies investigating the effect of training on the power of the alpha spectrum have shown that alpha power increases significantly after training [21, 33, 34], which probably means that the cortex is less involved in executive functions and more movements become automatic. Netz et al. (2019) showed that all physical exercises and dual tasks improve cognitive performance and improve brain neuroplasticity, and when the difficulty of exercise increases, neuroplasticity increases [51]. Studies have shown that athletes who have successfully returned to sports after an ACL injury have shown changes in their brain activity [2]. In this study, we expected that both training groups in standing on one leg with eyes opened and closed tasks in the post-test would achieve a higher symmetry in brain power of the alpha spectrum between two limbs. Also, the alpha activity is in an increasing trend. The results of our study in the post-test of the power of the alpha activity in the open eyes task in both the perturbation and standard training groups showed a decrease in average symmetry and there was no significant difference in the comparison of the two groups. Also, in the post-test of the alpha power activity in the eyes closed task, the perturbation training group showed excellent symmetry without change, but the standard training group showed almost asymmetry between the two limbs so that the brain's alpha power declined in standing on the right limb and had no change in standing on the left. According to Bazanova et al. (2013), alpha activity decreased when the eyes were open, which means increased cognitive load and more cortical activation [19]. Also, in external attention, which should be related to external visual events, the alpha power in the visual cortex decreases due to the increased excitability of the sensory cortex and increases the stimulus processing [48]. In line with the mentioned studies, the results of the

task of single-standing with opened eyes for two limbs showed a decreasing trend in both groups. According to Klimesch et al. (2012), alpha activity showed a decreasing trend when a task is in progress. Depending on the retrieval of information from long-term memory during the execution of the task, if the retrieved information is meaningfully stronger or more integrated, the alpha activity shows a greater decrease and the activation of the cerebral cortex occurs more [20]. In our study, in the task of single-leg standing with opened eyes, the decrease in alpha activity power can mean more semantic load and more cortex activation in balance control. Balance studies have shown that when a person closes their eyes due to a more challenging task and needs more assistance for balance control, the decrease in alpha activity is greater than when the eyes are open, which means greater activation of the cerebral cortex [13, 49, 50]. The results of our study did not show a change in the brain alpha power in the post-test of the comparison between the right and left limbs for the perturbation training group in single-leg standing with eyes closed, which is not in line with the results of the above studies. However, in the standard training group, the power of alpha waves did not change in the post-test of the left limb, but it showed a significant decrease for the right limb. In comparison, the perturbation training group had very good symmetry, while the symmetry obtained in this task was low for the standard training group. In summary, studies have shown that with the eyes open and in the presence of visual stimuli, the activity of the alpha power shows a decreasing trend [19]. The results of other studies that were conducted on the effect of exercise [21, 33, 34, 49], or studies done on the effect of internal attention [20, 48] on alpha power, were in contrast to studies on the more challenging closed eyes balance task [13, 21, 50].

5. Conclusion

In summary, ACLD individuals in the perturbation training group showed higher symmetry in relative alpha power in the performance of jump-landing and balance tasks compared to the standard training group. Mechanical perturbation training has a greater ability to improve the defective neuroplasticity created in the brain by increasing the symmetry of alpha band frequency in both limbs. The findings of the present study can be effectively used in training programs to prevent primary and secondary ACL injuries, as well as in training protocols after surgery. It is suggested that in order to achieve better treatment results in ACLD individuals and individuals with defective neuromuscular control, researchers design and study devices that are similar to real-world

sports events. Undoubtedly, EEG analysis in the frequency band domain is a promising tool to investigate the effect of training interventions on the recovery progress of ACLD individuals and their successful return to their active physical life.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of the Sports Sciences Research Institute (SSRI) (Code: IR.SSRC.REC.1399.095) and all participants accepted and signed the consent form to participate in this study before entering the study.

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Authors' contributions

All authors contributed equally to preparing all parts of the research.

Conflict of interest

The authors declared no conflict of interest.

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