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论文题目：The Effect of Physical Training on PIEZO2-Mediated Sense of Touch and Balance in Mice

The Effect of Physical Training on PIEZO2-Mediated Sense of Touch and Balance in Mice

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Abstract

The sense of touch and balance represent one of our five basic senses that allow us to actively interact with our external and internal world. In mammals including our humans, the PIEZO2 mechanoreceptor has been discovered to mediate the sense of touch and balance. Humans with loss-of-function mutations in PIEZO2 show defective sense of touch and balance. The discovery of PIEZO2 as the receptor for touch and balance has been recognized by the Nobel Prize in Physiology or Medicine in 2021. Given my strong passion and long-term practice in dancing, I am curious whether physical training might lead to improved balance capability via affecting the expression and function of PIEZO2. To test this hypothesis, we employed a set of physical training paradigms, including fatigue rod rotator task, grids stepping, and running wheel, to daily train experimental mice for a long period of 28-30 days. We tested touch sensitivity using the Von Frey assay and the balance capability using beam walking assay and rotarod test. Interestingly, we found that physical training remarkably increased the balance capability of the trained mice. The physically trained mice also show enhanced touch sensitivity, suggesting physical training-induced increase of PIEZO2 function. We detected the mRNA expression of PIEZO2 in sensory neurons using RT-PCR, but found no significant change of the mRNA level of PIEZO2 in mice subjected to physical training. Taken together, our data demonstrate that physical training can enhance the sensitivity of PIEZO2-mediated touch and balance. Future experiments will address whether physical training can increase the the protein expression or functional activities of PIEZO2, accounting for the physical training-induced enhancement of the sense of touch and balance performance. This study might suggest a physical training solution for improving balance capability for individuals with poor balance

or those professionals such as divers and dancers to further improve their balance capability.

Keywords:

PIEZO2, touch, balance, physical training

Table of Contents

Introduction

The sense of touch and balance

The sense of touch allows us to feel any objects, while the sense of balance allows us to keep our body positions and coordinate basic biological functions such as walking. Divers, figure skaters, dancers, and other professionals might require a high degree of ability to regulate their balance to complete high-quality diving, spinning, jumping, and other movements (Li et al., 2012). Given my strong passion in dancing, I often wonder how we can have the remarkable ability to sense the touch and balance? How my long-term practice of dancing helps me with greatly improved balancing capability so that I can perform those challenging dancing skills?

The sense of touch and balance requires the so-called sensory neurons whose cell bodies reside in the dorsal root ganglion to convey either a physical touch on the skin or a mechanical stretch of the muscle to electrical signal. "Proprioception is the sense of body and limb position and is transduced by proprioceptive sensory neurons. The information encoded by proprioceptors contributes to both unconscious and conscious sensations and is required for basic motor functions such as standing and walking" (Woo et al., 2015, 1757). Proprioception is a basic sense of our body and we often do not realize its existence and importance to our daily life. Here we refer proprioception the sense of balance.

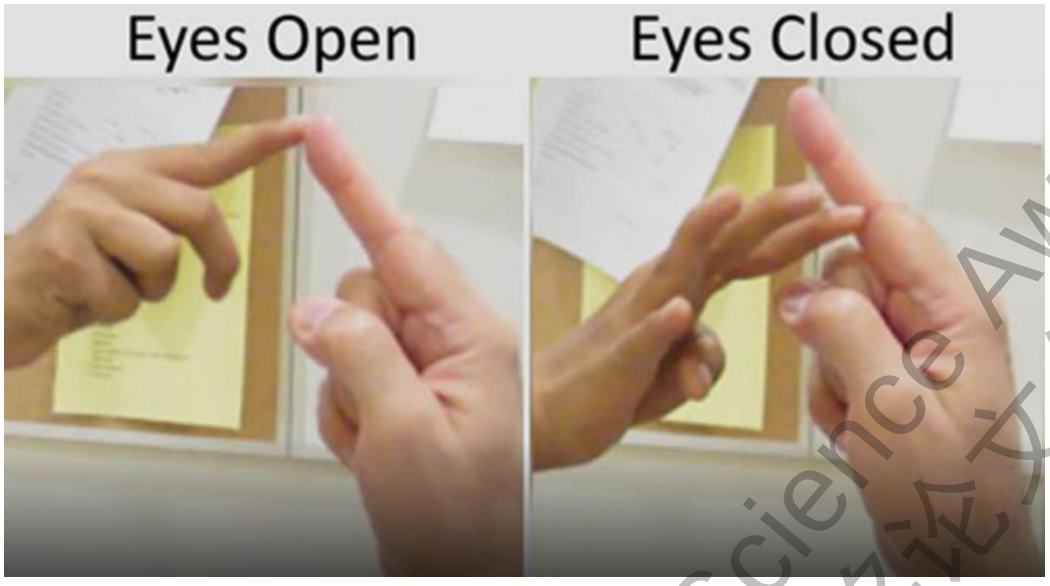
PIEZO2 as mechanoreceptor for touch and balance

To identify the mechanical force-sensing protein molecules that enable sensory neurons to sense force, the scientists selected glioma cells called Neuron 2A (N2A) for screening. They used the glass probe to give pressure to the N2A cell and detected the electrical signal. They hypothesized the force-sensing protein is a mechanosensitive cation channel that can sense and

respond to mechanical force and allow cations such as calcium and sodium ions to flow into the cell. The activation of such a mechanosensitive ion channel can convert mechanical force into excitation of sensory neurons and lead to the sense of touch and proprioception. After screening 72 candidate genes one by one, the scientists found that the knockout of the candidate gene Fam38A caused the elimination of the current. Researchers renamed the Fam38A gene as "PIEZO1" ---- a pressure-sensitive ion channel gene. PIEZO1 has a homologous gene called PIEZO2.

PIEZO proteins represent a novel class of ion channels distinct from other known ion channel families. Importantly, PIEZO2 is expressed in dorsal root ganglion sensory neurons and mediate mechanically activated cationic currents (Coste, 2010). By knocking out PIEZO2 in sensory neurons, it has been demonstrated that PIEZO2 functions as the mechanoreceptor for touch and balance.

In a study published in 2016 (Chesler, 2016), it reports that "consistent with these findings, humans with loss-of-function mutations in PIEZO2 display profound deficits in touch sensation, including texture discrimination, hair deflection as well as tactile and vibration sensitivity. Additionally, humans with loss-of-function mutations in PIEZO2 tend to lose the ability to walk properly when their eyes are covered to block their vision. "The patient's version of PIEZO2 may not work, so their neurons cannot detect touch or limb movements." These human studies have convincingly demonstrated the principal role of PIEZO2 as receptors for sensing touch and balance (Fig. 1).



https://www.genengnews.com/news/genetic-basis-of-sixth-sense-revealed/

Fig. 1 The patient carrying a loss-of-function mutation in the Piezo2 gene cause their sensory neurons unable to sense the touch and body positions, and consequently lose the ability to detect touch or limb movements.

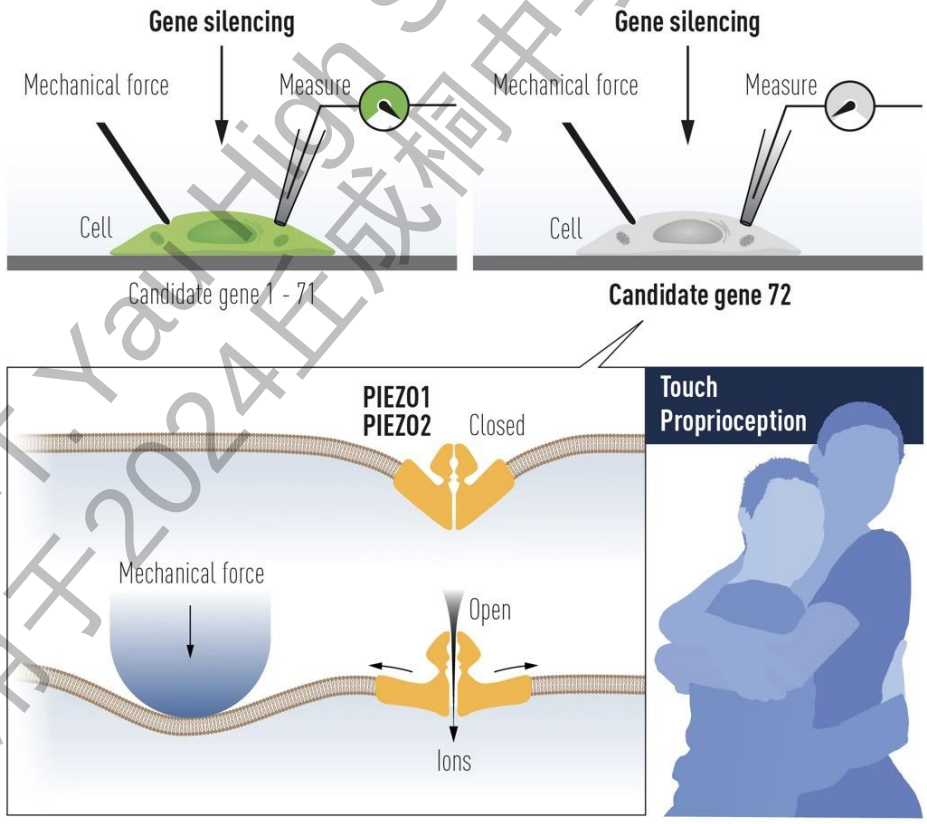


Fig. 2 The discovery of PIEZO2 as mechanoreceptor for touch and balance

(proprioception) (adopted from https://www.nobelprize.org/prizes/medicine/2021/press-release).

The discovery of PIEZO2 as mechanoreceptor for touch and balance has been recognized by the Nobel Prize in Physiology or Medicine in 2021 (Fig. 2), and the discover Dr. Ardem Patapoutian has shared the Nobel Prize with Dr. David Julius who discovered the receptor for temperature.

Key research question and working hypothesis

In our daily lives, we can recognize many people who have a strong balanced capability. For example, talented dancers and athletes can spin for a long time and stop in a stable and elegant position through high-strength training for an extended period. How can physical training improve the balance capability? Given that PIEZO2 serves as a balance receptor for conferring balance capability, we hypothesize that the level of expression and function of PIEZO2 might serve as a key index for reflecting the poor or strong balance capability, and that physical training such as spinning and jumping might affect the balance capability by changing the expression and function of PIEZO2. However, there is little research focusing on the specific relationship between the expression and function of PIEZO2 and the balance capability. Therefore, we aim to address this knowledge gap by setting up physical training protocols in mice and examining the training-induced effect on balance capability and on the expression and function of PIEZO2.

Methodology

Our study is a quantitative research, using experiments that relate to the investigation of PIEZO2's functioning to approach the research problem.

Phase 1

In the first phase of the experiment, we chose 20 male mice as the experimental objects and divided them into two groups – the control group without training and the training group that is subjected to physical training. We began our original test of each balance task after letting the mice adapt the equipment for 2-3 days. We used the von Frey test, beam walking, and grid stepping to attest the two groups' original balance capacity.

Phase 2

After the initial test for the balance capability before training, we started the training for the training group which **lasted for about 28-30 days**. There are 2 tasks for each day.

Fatigue rod rotator task: 10 minutes/day for the training group numbered from 6A-10A. Put the mice onto the equipment and let them walk on the running beam. Put it back onto the equipment again if it falls until the time ends.

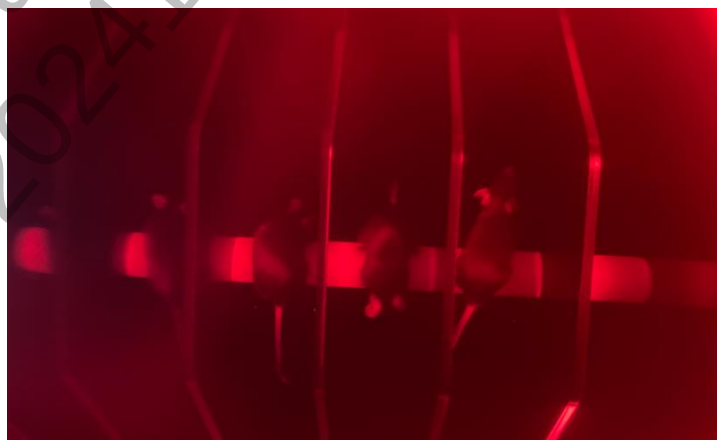


Fig. 3 The fatigue rod rotator task.

Grids stepping: 10 minutes/day for the training group 6A-10A. Put the mice onto the grid of a size of 300*300 mm until the time ends.

Fig.4 The grid stepping task

Running wheel: we put the running wheel into each training group mice's cage. In this way, the training group will be able to take extra physical training independently even at the time without monitoring.

Phase 3

At the end of the training period, we tested the 2 groups' balance capacity using 3 different assays.

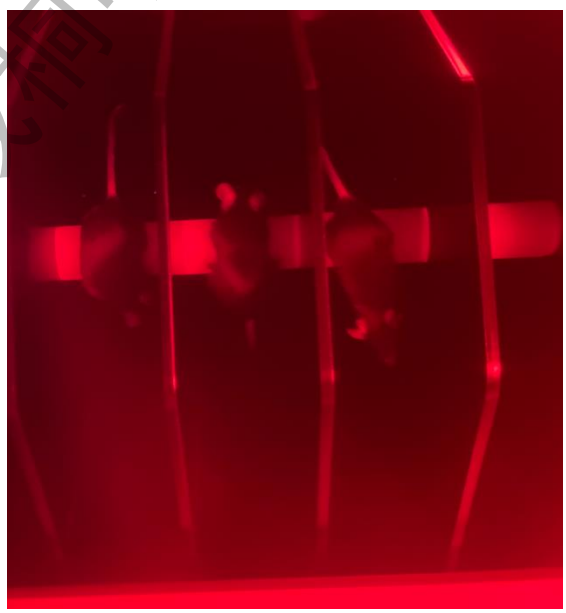
Beam walking: prepare 2 sticks with different degrees of thickness (12mm and 6mm), and let the mice adapt the equipment for 1 day. Put the two groups of mice, including 1A - 5A (control group) and 6A - 10A (training group) on the stick and let them walk through the whole stick. Use the camera to record the time the mouse cost to complete the distance.

Von Frey test: Let the mice adapt the equipment for 1 day. Put the two groups of mice into the cave that separates them into individuals. Use the von Frey equipment to touch the mice's feet with different fitments (0.04 - 2.00g), each flighting should be tested 6 times and calculate the average von Frey.

Rotating rod test: Let the mice adapt the equipment for 1 day. Put the two groups of mice onto the rod and record the time that they fall for the first time.

(A)

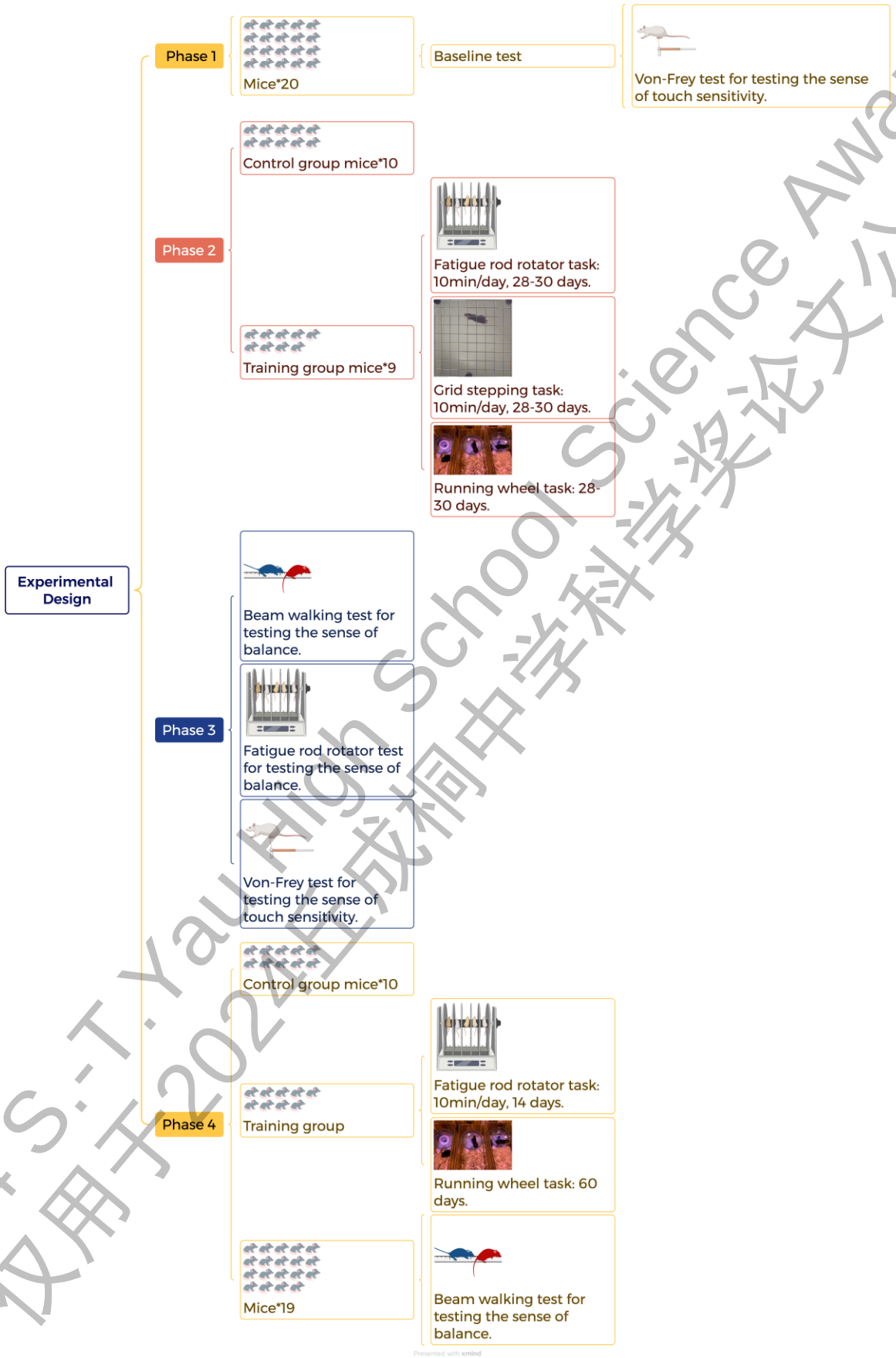
(B)



(C)

Fig. 5 The experiments that represent the different behaviors of the control and trained mice. (A) shows the beam walking test that records the speed of the mice completing the

distance. (B) shows the process of the von Frey experiment, expressing the touch sensitivity of the mice. (C) shows the process of the rotating rod test for the balance capability.



Phase 4

To ensure the two groups of mice retain the same experiment results as 2 months ago, we repeated the training tasks for 14 days and the beam walking test to measure the behavioral performance of both the control and training groups.

As we attain the result to compare the two groups' balance capability, we attest to the PIEZO2 expression of each group to indicate the effect of physical training.

Isolation of mouse dorsal root ganglion (DRG) neurons:

1. Anesthetize the animal with 5% isoflurane.

2. Once under deep anesthesia, sacrifice the animal via decapitation by using large surgical scissors and try to avoid damaging the cervical vertebra.

3. Remove the dorsal skin and excess fatty tissue of the animal.

4. Isolate the vertebral column, and soak the vertebral column in the DPBS solution.

5. Use fine scissors to cut away useless muscles and fatty tissue.

6. Sectioning the vertebral column

    a. Show the view of the ventral side of the vertebral column.

    b. Use the fine dissecting scissors to cut through the opening of the cervical and remove the top half of the vertebral column.

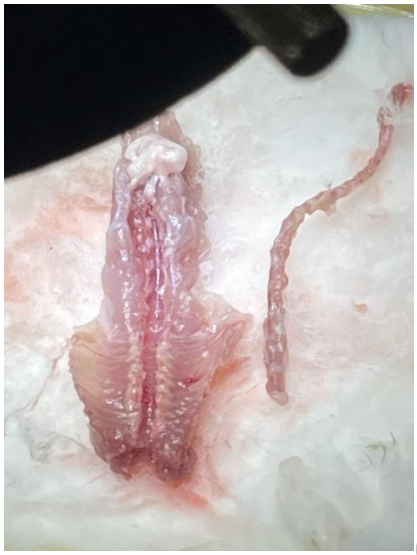


Fig. 6 Sectioning of the vertebral column.

7. Select DRG

a. Peel the spinal cord tissue away from the vertebral column, and the DRG should be exposed.

b. Gently lift the DRG out by using the forceps and place the DRG into the HBSS collection tube kept on ice.

c. Reduce the extra PBS solution

Phase 5

Real-time polymerase chain reaction

1. Use liquid nitrogen to grind the DRG Tissue.

2. RNA preparation through RNA extraction kit (UE-MN-MS-RNA-50).

3. Detection of isolated RNA concentration and purity by NanoDrop.

4. Reverse transcription of 2μg of RNA in a 20μl reaction system using the Reverse Transcription Kit.

a. 0.1-5μg RNA+1 μl random primer, total volume 12μl, incubate at 65°C for 5min and let stand on ice for 5min (The primers for RT-PCR are shown in Table 1).

b. According to the system in Table 2, add components and mix well. The total volume of 20μl, invert at 42℃ for 1h; terminate at 70 ℃ for 5 min, and store at -20 ℃

Table 1. RT-PCR Primer Sequence

Primer Name	Primer Sequence（5'-3'）
mPiezo2-qPCR-F	ACTATGCAAGGTTGTTTGGGA
mPiezo2-qPCR-R	CACCCTCATCTTCCTTCGCC
β-actin-Forward	GATCAAGATCATTGCTCCTCCTG
β-actin-Forward	AGGGTGTAAAACGCAGCTCA

Table 2. Reversion System

Component	Volume
5 X Reaction Buffer	4 μl
RiboLock Rnase Inhibitor (20 U/μl)	1 μl
10 mM dNTP Mix	2 μl
RevertAid M-MuLV RT	1 μl
Total volume	20 μl

5. Add 1.5μl cDNA template (~25 ng), 3μl forward primer (1 μM), 3μl reverse primer (1 μM), 7.5 μl SYBR for the RT-PCR system.

6. Let the expression of Piezo2 cf. be the internal reference of β-actin, and start the RT-PCR program.

Table 3. RT-PCR Program

Temperature	Time	Cycle Number
50℃（Hold stage）	2 min	1
95℃（Hold stage）	10 min	1
95℃（PCR stage）	15 s	40
60℃（PCR stage）	60 s	
95℃（Melting curve）	15 s	1
65℃（Melting curve）	15 s	1

7. Use ViiA™ 7 Real-Time PCR software to analyze the result and gene expression was analyzed using the 2-ΔΔCT method (Livak and Schmittgen et.al, 2001).

Results

Exercise training improves balance capability

To assess the balance and limb coordination of male mice, we employed three established behavioral experiments: the von Frey experiment, the rotating rod test, and the beam walking test for mice from both the control and training group mice. All tests were performed longitudinally during mouse maturation from adolescent (6, 8, and 10 weeks) to young adult age (12 weeks).

Beam walking test

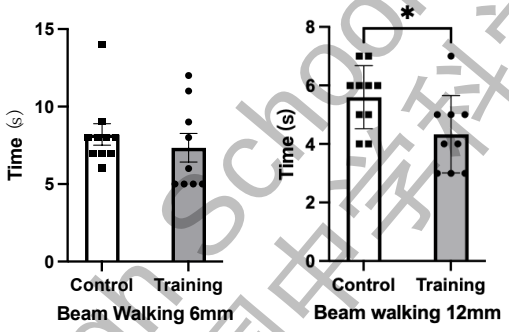


Fig. 6 Beam walking test in two beams with different thicknesses (6 mm and 12 mm). The speed of completing the distance has been calculated and analyzed. The shade bars indicate the mean speed of each group.

The time required for the control and trained mice to cross the beam with a diameter of either 12 mm or 6 mm was recorded (please see the Methodology for details). The less time the mice need to cross the beam, the better the balance capability of the mice.

During the first test, the statistical difference between the control and training groups was analyzed using an unpaired t-test. When tested on the 12mm beam, the training group spent significantly less time crossing the 12mm beam compared to the control mice (figure 5). Thus, the training group performed better than the control group. On the other hand, when

assayed on the even tougher 6mm beam test, there was no significant difference between the control and training mice, suggesting that the training-induced improvement of the training group was not strong enough to outcompete the control group in the more challenging 6 mm beam test.

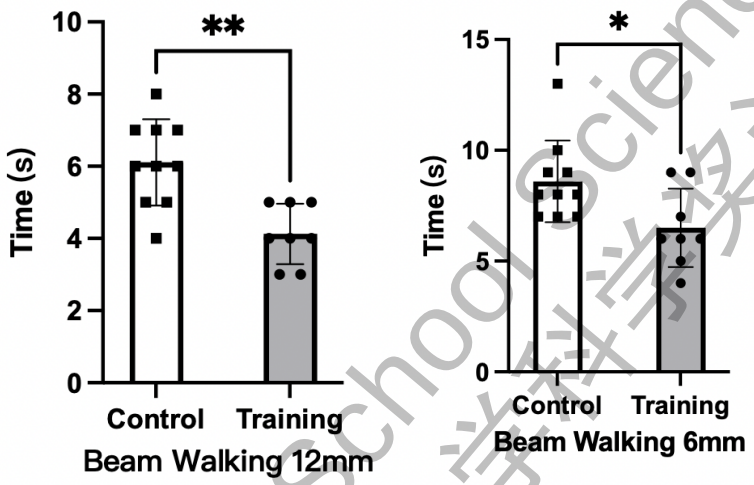


Fig. 7 To ensure the two groups of mice maintained the same balance capability in the long-term as the first time's Beam Walking Test result, we repeated the experiment after two months.

However, we repeated the test after two months which is based on one week's rotating rod training and extra running wheel exercise. In the second round of testing, the result of both the 6mm and 12mm beams showed a significant difference between the control and training groups. Therefore, the training group had a long-lasting increasing balance capability compared with the control group.

Rotating Rod test

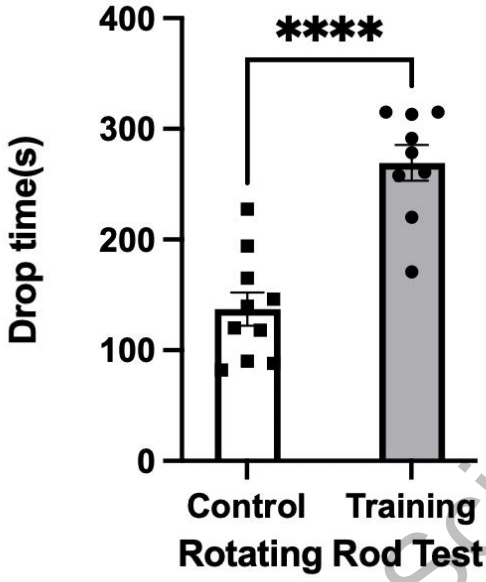


Fig. 8 A comparison of the ability of balance measured by the time mice maintained balance on the running wheel. The shaded bars represent the average time of dropping.

Both tests in different periods show a significant difference between the control and training groups. This was due to the adaptational training for the training group before the previous rotating rod test which reduced the anxiety of the mice in the training group and led to a better performance than the mice in the control group during the task. The result shows a remarkable improvement in the training group based on the statistical t-test for the mean of difference (paired t-test), as suggested by a significant difference in the training group's data: a greater time-keeping balance on the rod tester instead of dropping after 90 days of exercise.

The function of PIEZO2 is increased by exercise training

Von Frey test:

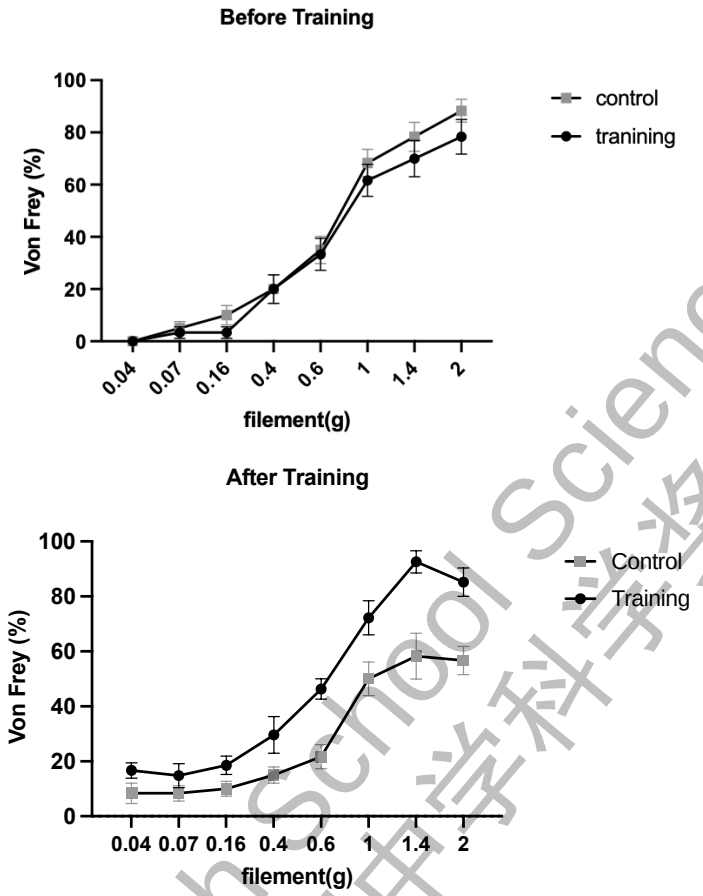


Fig. 9 The touch sensitivity of mice is increased by exercise tasks. The dot shows the percentage of mice with obvious response to the filament.

The result of the Von Frey experiment reflects the PIEZO2-mediated touch sensitivity of mice. During the physical training process, a significant difference has been obtained between the two periods of the training group. The result shows a positive influence on the touch behavior of the training mice in the test. Thus, it suggests that training might increase the function of PIEZO2 and consequently result in the enhanced touch sensitivity of the training group.

Real-Time PCR

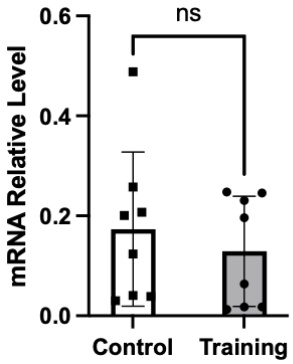


Fig. 10 Fluorescent signals are captured throughout the PCR process, and changes in the volume of amplification products are monitored in real-time in each cycle, and the samples measured are finally quantitatively analyzed by the standard curve 9 and CT values.

To examine whether the increased function of PIEZO2 is due to its increased mRNA expression in DRG neurons, we carried out RT-PCR experiments and found no significant difference of mRNA level of PIEZO2 in DRG between the control and training groups. The result indicates that physical training did not strongly affect the mRNA expression of PIEZO2. However, given the apparently enhanced PIEZO2-mediated touch sensitivity in the training group, we reason that the protein level or activity of PIEZO2 might be affected by physical training. Future experiments will continue to test the possibility. If no change in PIEZO2 expression, we might consider other factors such as muscle content, and physicological impacts that might cause the exercise-induced change of the mice's balance capability.

Discussion

Based on the knowledge that PIEZO2 serves as mechanoreceptors for touch and balance, we have tested the hypothesis that physical training might increase the balance performance by affecting the expression and function of PIEZO2. In support of the hypothesis, we found that physical training can improve the mice's performance of balance tasks. Using three distinct assays, including beam walking, rotating rod test, and beam walking tasks, we consistently observed the physically trained group of mice had better balance performance. The result suggests a higher sensitivity of the trained mice compared to the control mice after continuing 60 days of training. Interestingly, the physically trained group of mice also had better responses in von Frey test, which might suggest sensitized touch sensitivity. Given that touch is mediated by PIEZO2, these data suggest that physical training increases the function of PIEZO2. However, RT-PCR detection of the mRNA expression of PIEZO2 in DRG neurons did not show a significant difference between the control and trained groups of mice. These results collectively indicate that physical training might be more likely to affect the protein level and function of PIEZO2, instead of its mRNA expression, which requires additional future studies.

The present findings add substantially to our understanding of the relationship between exercise-induced balance and the role of PIEZO2. The entire experiment has been separated into two fields: the behavioral and molecular perspectives. The results show a positive impact that exercise tasks can improve the balance capability. While much of the previous literature has focused on how the function of PIEZO2 affects limb coordination, regular movements, and balance capability, there have been few studies that examine the external factors that impact the role of PIEZO2. Thus, there is scope for further research which is spending effort to provide

a deeper discovery of the interaction between PIEZO2 and exercise-induced balance and can relate to people's daily exercise and movement regulation.

The study also has some limitations. In the experiment, we chose to use the von Frey experiment to reflect the alternation of PIEZO2 function by showing the sensitivity under different levels of forces. This kind of behavioral experiment will be affected by many factors such as the mice's anxiety, movements, and other disturbances, and the researcher's personal bias while determining the response of the mice after feeling touch or pain, and the different strengths the researchers use during each round of the experiment. As a result, the von Frey experiment only can represent the change in the function of PIEZO2 in an indirect method, which may influence the results that conclude the relationship between exercise and PIEZO2 expression. Additionally, due to time limits, the training tasks have not been continuous. The gap between two beam walking tests may cause the PIEZO2 activity to alter and affect the final examination. Therefore, we may continue to study this issue and consider a wider range of possibilities and methods in depth.

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The inspiration for this research topic was a common activity in our lives - dancing. During the dance club's training, I found that everyone showed a different level of balance ability and it reminded me of the PIEZO2 mechanoreceptor that I've learned before, after communicating with several faculty members, they guided the complete research without compensation. Without their support, I would not be able to start a complete research by myself. It is my honor to collaborate with these great teachers and scientists, from this experience, I've

learned about the spirit of a scientist - sacrifice, preservice, presence, and creativity from all of them. They encouraged me to preserve the project and have the confidence to once become a "true scientist" in this activity.

We went through many issues during each period. For me, the biggest difficulty was overcoming the fear of operating the surgery by myself. I was nervous about injecting the anesthetic as the mouse always reacts intensely to the needle. During the first try, I injected the anesthetic under the skin instead of into the abdominal cavity, and the anesthetic remained under the skin tissue and formed a large swelling. Though I was truly resistant to the operation of this step, I practiced again and again, using my fingers to press the mouse from its back to the cervical vertebrae and my hand controlling the whole body to expose its abdomen, then quickly insert the needle from the mouse's midline against the right abdominal cavity. Practice makes perfect, I finally figured out the most appropriate way to practice the operation.