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## **Title: Effect of Yi-Nao-Jie-Yu Prescription on Post-Stroke Depression in Rats using Middle Cerebral Artery Occlusion Combined with Behavioral Restraint**

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## **Author Questionnaire**

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

### **Current Protocol Length**

Number of Steps: 12

Number of Shots: 29

# Introduction

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*Videographer: Obtain headshots for all authors available at the filming location.*

## INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

1.1. **Yuankai Su:** We developed a rat model of post-stroke depression and evaluated whether the Chinese medicine Yi-Nao-Jie-Yu alleviates depression-like behaviors.

~~1.1. 宿元开: 我们建立了一种脑卒中后抑郁的大鼠模型, 并评估了中药颐脑解郁方是否能缓解抑郁样行为。~~

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~1.1.1. 采访: 指定专家以上述陈述进行采访式拍摄, 视线略微偏离镜头。~~

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

1.2. **Yonghao Sun:** Existing models inadequately combine stroke and chronic stress; this protocol integrates artery blockage, prolonged restraint, and isolation to better replicate post-stroke depression.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

## CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

1.3. **Yonghao Sun:** This protocol lets us measure behaviors linked to depression. We record time spent floating (despair), sugar water intake (loss of interest), and activity in an open field.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Yuankai Su:** The key challenge is ensuring precise, humane execution, requiring accurate suture placement and secure restraint without compromising respiration or animal welfare.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Yonghao Sun:** Future work can study how this Chinese medicine works in the brain. We can also test if combining it with other treatments, like rehabilitation or Western drugs, is more effective.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

***Videographer: Obtain headshots for all authors available at the filming location.***

**Ethics Title Card**

This research has been approved by the Animal Care and Use Committee at Beijing University of Chinese Medicine

# Protocol

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## 2. Assessment of Poststroke Depression and Antidepressant Interventions in a Rat Model Using Behavioral Tests

**Demonstrator:** Yonghao Sun, Yuankai Su

2.1. To begin, perform behavioral restraint and isolation housing on post-middle cerebral artery occlusion day 7 to avoid the acute stroke phase [1]. Prepare the custom T-shaped restraint platform [2-TXT].

2.1.1. Talent preparing animals for restraint and isolation housing on post-middle cerebral artery occlusion day 7.

2.1.2. Talent assembling the T-shaped restraint platform using measured components.  
**TXT: Dimensions: Base: 20 cm x 10 cm x 2.8 cm;  
Restraint area: 22 cm x 6.6 cm**

2.2. Ensure the restraint area contains slots for fixing the rat's head and limbs and three adjustable straps to fit different rat sizes [1].

2.2.1. Talent inserting adjustable straps and aligning the slots designed for the head and limbs on the platform.

2.3. At any random 2-hour window each day, secure each rat to the platform using the slots [1] and strap then adjust the tightness to prevent struggling without causing respiratory suppression [2-TXT].

2.3.1. Talent securing a rat on the restraint platform.

2.3.2. Shot of the straps being carefully adjusted. **TXT: Restrain for 2 h every day for 7 days**

2.4. Transfer rats initially grouped in 5, to individual cages at the start of behavioral restraint [1]. Maintain single housing until behavioral restraint is completed on post-middle cerebral artery occlusion day 14 [2].

2.4.1. Talent moving each rat from a shared cage to individual single housing cages.

**AND**

TEXT ON PLAIN BACKGROUND:

Groups of 5

PSD: Poststroke Depression

FXT: Fluoxetine Hydrochloride

YNJP: Yi-Nao-Jie-Yu Prescription

*Video Editor: please play both shots side by side in a split screen*

- 2.4.2. Shot of singly housed rats in individual cages.
- 2.5. For the sucrose consumption test, randomly select 6 rats from the groups at 2, 4 and 8 weeks post-middle cerebral artery occlusion [1]. Fast and deprive the rats of water for 24 hours before the sucrose consumption test [2]. Ensure the testing room temperature is maintained at 23 plus or minus 2 degrees Celsius and the lighting matches the housing environment [3].
- 2.5.1. Shot of random rats being selected from labeled cages with rats at 2-8 weeks post MCAO.
- 2.5.2. Talent removing food and water from the cages.
- 2.5.3. Talent checking the testing room thermostat and lighting to confirm environmental consistency.
- 2.6. Then place two 150-milliliter bottles in each cage, one containing tap water and the other containing 1 percent sucrose solution [1]. After 1 hour of free drinking, record the volumes of sucrose solution and water consumed [2].
- 2.6.1. Talent placing the two labeled bottles in each rat's cage.
- 2.6.2. Talent measuring and documenting the volume of liquid consumed from each bottle after 1 hour.
- 2.7. To calculate sucrose preference, use the given formula [1]. Swap the positions of the two bottles halfway through the drinking period to eliminate position bias [2].
- 2.7.1. TEXT ON PLAIN BACKGROUND:  
$$\text{Sucrose preference (\%)} = (\text{Volume of sucrose solution consumed} / \text{Total volume consumed}) \times 100\%$$
- 2.7.2. Talent switching the positions of the two bottles during the 1-hour test.
- 2.8. For the open-field test, set up a black square box ~~with the floor divided into 25 equal grids~~ [1]. Keep the testing room quiet and dimly lit [2].
- 2.8.1. Talent placing the open-field apparatus on the floor ~~and ensuring the grid layout is clear.~~
- 2.8.2. Talent turning off overhead lights and closing doors to maintain a dim, quiet environment.
- 2.9. Place each rat in the center of the open-field box and start a 5-minute timer [1]. Record the rat's horizontal activity movement within the box [2-TXT], and vertical activity as the rearing with the forepaws off the floor or climbing against the wall [3]. Optionally, record grooming and other typical behaviors for additional observation [4].
- 2.9.1. Talent places a rat in the central area of the box and starts a stopwatch.
- 2.9.2. Shot of rat showing horizontal movement within the box. **TXT: Use handheld counters for counting**

- 2.9.3. Shot of rat performing vertical activity
- 2.9.4. Talent observes and records grooming and other typical behaviors.  
*Videographer: Please note that this is an optional step*
- 2.10. To conduct the forced swim test, first fill a glass cylinder with water [1-TXT]. Adjust the water temperature to 25 plus or minus 1 degree Celsius [2].
  - 2.10.1. Talent filling the glass cylinder with water and checking the depth. **TXT: Ensure water level is high enough that rat cannot touch the bottom with its feet**
  - 2.10.2. Talent using a thermometer to verify the water temperature.
- 2.11. Place each rat in the cylinder and let it swim for 5 minutes [1]. Record the total immobility time during the 5 minutes [2]. Define immobility as passive floating with only minimal movements to keep the nose above water [3] and activity as limb paddling or wall-scratching [4].
  - 2.11.1. Talent gently placing a rat into the cylinder.
  - 2.11.2. Talent monitoring and timing the duration of immobility.
  - 2.11.3. Shot of animal showing passive floating.
  - 2.11.4. Shot of animal showing limb padding or wall-scratching.
- 2.12. After the test is complete, remove the rat from the cylinder [1], dry it with a towel [2], and place it under a heating lamp set to 30 plus or minus 2 degrees Celsius for 30 minutes to prevent hypothermia [3].
  - 2.12.1. Shot of rat being removed from the cylinder.
  - 2.12.2. Talent drying the rat with a towel.
  - 2.12.3. Talent positioning dried rat under a heating lamp.

# Results

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## 3. Results

- 3.1. At 4 weeks, the immobility time of post-stroke depression rats in the Forced Swim Test was significantly higher than that of stroke group rats [1].
  - 3.1.1. LAB MEDIA: Figure 6. *Video editor: Highlight the bar for “PSD group” and “Stroke group” at “4 Weeks” in the “Forced Swim Test” graph.*
- 3.2. At both 4 and 8 weeks, the sucrose preference of PSD rats in the Sucrose Preference Test was significantly lower than that of the stroke group rats [2].
  - 3.2.1. LAB MEDIA: Figure 6. *Video editor: Highlight the bar for “PSD group” and “Stroke group” at “4 Weeks” and “8 Weeks” in the “Sucrose Preference Test” graph.*
- 3.3. The YNJYP (Y-N-J-Y-P) treatment significantly reduced the immobility time of PSD (P-S-D) rats at both 4 and 8 weeks [1]. YNJYP treatment also significantly increased their sucrose preference at both time points [2].
  - 3.3.1. LAB MEDIA: Figure 6. *Video editor: Highlight the bar for “TCM group” at “4 Weeks” and “8 Weeks” in the “Forced Swim Test” graph.*
  - 3.3.2. LAB MEDIA: Figure 6. *Video editor: Highlight the bar for “TCM group” at “4 Weeks” and “8 Weeks” in the “Sucrose Preference Test” graph.*
- 3.4. At 8 weeks, the rearing frequency of PSD rats was significantly lower than that of the sham-operated group in the Open Field Test [1].
  - 3.4.1. LAB MEDIA: Figure 6. *Video editor: Highlight the bar for “PSD group” and “Sham operated” at “8 Weeks” in the “Rearing Frequency” graph.*
- 3.5. At 2 weeks, the locomotor activity of PSD rats was significantly lower than that of the stroke group in the Open Field Test [1].
  - 3.5.1. LAB MEDIA: Figure 6. *Video editor: Highlight the bar for “PSD group” and “Stroke group” at “2 Weeks” in the “Locomotor Activity Counts” graph. (top right graph)*
- 3.6. At 8 weeks, the rearing frequency of both the TCM group and the conventional medicine group was significantly higher than that of the PSD group [1]. The locomotor activity of the TCM (T-C-M) group and the conventional medicine group was significantly higher than that of the PSD group at both 2 and 8 weeks [2].
  - 3.6.1. LAB MEDIA: Figure 6. *Video editor: Highlight the bar for “TCM group” at “8 Weeks” in the “Rearing Frequency” graph.*
  - 3.6.2. LAB MEDIA: Figure 6. *Video editor: Highlight the bar for “TCM group” and*

*Conventional medicine group” at “2 Weeks” in the “Locomotor Activity Counts” graph.*

Pronunciation Guide:

Yi-Nao-Jie-Yu

Pronunciation link: No confirmed link found

IPA: /jiː naʊ dʒiː juː/

Phonetic Spelling: yee·now·jee·yoo

Post-Stroke Depression

Pronunciation link: <https://www.merriam-webster.com/dictionary/post-stroke>

IPA: /'pəʊst ˌstroʊk dɪˈpreʃən/

Phonetic Spelling: pohst·strohk·dih·preh·shuhn

Middle Cerebral Artery Occlusion

Pronunciation link: <https://www.merriam-webster.com/dictionary/occlusion>

IPA: /'mɪd.l̩ səˈriː.brəl ˈɑːr.tə.ri əˈkluː.ʒən/

Phonetic Spelling: mid·luh·suh·ree·bruhl·ahr·tuh·ree·uh·kloo·zhuhn

Behavioral Restraint

Pronunciation link: <https://www.merriam-webster.com/dictionary/restraint>

IPA: /bɪˈheɪ.vjər.əl rɪˈstreɪnt/

Phonetic Spelling: bih·hay·vyur·uhl·rih·straynt

Fluoxetine Hydrochloride

Pronunciation link: <https://www.merriam-webster.com/dictionary/fluoxetine>

IPA: /fluːˈɑːk.səˌtiːn haɪˈdrɒˌklɔːr.aɪd/

Phonetic Spelling: floo·ahk·suh·teen·hy·droh·klor·ide

Sucrose Preference Test

Pronunciation link: <https://www.merriam-webster.com/dictionary/sucrose>

IPA: /'suː.kroʊs ˈprɛf.rəns tɛst/

Phonetic Spelling: soo·krohss·preh·fruhns·test

Forced Swim Test

Pronunciation link: <https://www.merriam-webster.com/dictionary/forced>

IPA: /fɔːrst swɪm tɛst/

Phonetic Spelling: forst·swim·test

Open-Field Test

Pronunciation link: <https://www.merriam-webster.com/dictionary/open-field>

IPA: /'oʊ.pən fi:ld tɛst/

Phonetic Spelling: oh·puhn·feeld·tɛst

Locomotor Activity

Pronunciation link: <https://www.merriam-webster.com/dictionary/locomotor>

IPA: /,loʊ.kə'moʊ.tər æk'tɪ.və.ti/

Phonetic Spelling: loh·kuh·moh·tur·ak·tiv·uh·tee

Hypothermia

Pronunciation link: <https://www.merriam-webster.com/dictionary/hypothermia>

IPA: /,haɪ.poʊ'θɜ:r.mi.ə/

Phonetic Spelling: hy·poh·thur·mee·uh

Antidepressant

Pronunciation link: <https://www.merriam-webster.com/dictionary/antidepressant>

IPA: /,æn.tɪ.dɪ'pres.ənt/

Phonetic Spelling: an·tee·dih·preh·suhnt

Immobility

Pronunciation link: <https://www.merriam-webster.com/dictionary/immobility>

IPA: /,ɪ.moʊ'bɪ.lə.ti/

Phonetic Spelling: ih·moh·bil·uh·tee

Cerebral

Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebral>

IPA: /sə'ri:brəl/

Phonetic Spelling: suh·ree·bruhl

Occlusion

Pronunciation link: <https://www.merriam-webster.com/dictionary/occlusion>

IPA: /ə'klu:ʒən/

Phonetic Spelling: uh·kloo·zhuhn

Restraint

Pronunciation link: <https://www.merriam-webster.com/dictionary/restraint>

IPA: /rɪˈstreɪnt/

Phonetic Spelling: rih·straynt