FINAL SCRIPT: APPROVED FOR FILMING

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Title: Network Pharmacology and Validation of the Antidepressant Mechanisms of Qiangzhifang in a Chronic Restraint Stress-Induced Depression Rat Model

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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? **Yes, all done**

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 19
Number of Shots: 38

Introduction

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

1.1. **Wanqing Ren:** This research aims to investigate how Qiangzhifang acts against depression, identify its key active ingredients, and understand their mechanisms of action.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.4.1.*

What research gap are you addressing with your protocol?

1.2. **Wanqing Ren:** This study fills the gap in understanding the specific mechanisms and active components of Qiangzhifang for treating depression.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 6.1.4.*

What research questions will your laboratory focus on in the future?

1.3. **Tongzheng Liu:** We will study how Qiangzhifang affects the immune system to fight depression and explore its potential new therapeutic applications.

1.3.1. 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 Experiment Ethics Committee of Shandong University of Traditional Chinese Medicine

Protocol

2. Establishment of a Chronic Restraint Stress (CRS) Rat Model and Drug Intervention

Demonstrator: Wanqing Ren

2.1. To begin, construct the rat restraint device by selecting a transparent plastic tube with a diameter and length suitable for the rat's size, allowing the rat to stand and turn inside while preventing escape **[1]**. Using an electric soldering iron hole puncher, create holes on the sides of the plastic tube and on the lid to ensure proper air circulation **[2]**.

2.1.1. WIDE: A shot of the transparent plastic tube and the talent standing beside it.

2.1.2. Talent using an electric soldering iron hole puncher to make holes on the tube's sides and lid.

2.2. Divide the rats into four groups, ensuring each group consists of 10 rats **[1]**.

2.2.1. TEXT on PLAIN BACKGROUND:

Rat groups:

1) The control (CON) group
2) The model (CRS) group
3) The Qiangzhifang (QZF) group
4) The fluoxetine (F) group

2.3. One hour after daily intragastric administration of the drug, gently place the rats in the restraint devices, excluding those in the control group, and ensure they are in a comfortable position **[1-TXT]**.

2.3.1. Talent placing a rat into the restraint device. **TXT: Deprive all rat groups of food and water during each 6 h restraint period; After restraint, provide ample food and water uniformly for 28 days**

2.4. Administer fluoxetine at 2.7 milligrams per kilogram per day to the fluoxetine group and Qiangzhifang at 2 grams per kilogram per day to the Qiangzhifang group via gavage, using a dosage volume of 1 milliliter per 100 grams of body weight for both solutions **[1-TXT].**

2.4.1. Talent administering the solution to a rat via gavage. **TXT: Provide the control and CRS groups with equivalent normal saline for single-variable control**

3. Sucrose Preference Test (SPT)

Demonstrator: Wanqing Ren

3.1. Fill the drinking bottles of the experimental animals with pure water or a 1 percent aqueous sucrose solution, placing each solution in a separate bottle **[1]**. Then, weigh the bottles **[2]**.

3.1.1. A shot of the drinking bottles of the experimental animals filled with pure water and 1 percent aqueous sucrose solution.

3.1.2. Talent weighing the bottles.

3.2. Place one bottle of sucrose solution and one bottle of pure water at each rat cage's water intake, one on the left and one on the right, for free access to drinking **[1-TXT]**. After 30 minutes, switch the positions of the bottles to avoid side preference **[2]**.

3.2.1. Talent placing one bottle of sucrose solution and one bottle of pure water in the rat cage's water intake, ensuring one is on the left and the other on the right. **TXT: Deprive the rats of food and water for 24 h before the experiment**
Authors: Please label the water bottles.
Videographer: Please make sure the labels on the bottles are visible in the frame.

3.2.2. Talent switching bottle positions.

3.3. After 1 hour, remove all water bottles **[1]**, weigh them promptly to measure the consumption values for both solutions **[2-TXT]**. Then, calculate the weekly sucrose preference ratio using the given equation **[3]**.

3.3.1. Talent removing bottles.

3.3.2. Talent placing the bottle on a digital balance to measure weight. **TXT: Solution consumption = Bottle weight before experiment – Bottle weight after experiment**

3.3.3. TEXT on PLAIN BACKGROUND:

Sucrose preference value

$$= \frac{(\text{Consumption of sucrose solution (g)})}{[(\text{Consumption of sucrose solution (g)}) + (\text{Consumption of pure water (g)})]} \times 100\%$$

3.4. Weigh the rats weekly at a fixed time of 7 in the morning to monitor changes in body weight **[1]**.

3.4.1. Talent placing each rat on a digital scale and recording the weight.

4. Open-Field Test (OFT)

Demonstrator: Tongzheng Liu

4.1. Adjust the lighting in the open field box for even distribution **[1]** and clear visibility of the rats in the tracking software **[2]**.

4.1.1. Talent adjusting lights around the open field box.

4.1.2. The visibility of the rats being checked through the tracking software interface. *Videographer: Please record the screen for this shot.*

4.2. Use the video tracking and analysis system to divide the bottom surface of the open field box into nine equal square grids, with eight grids adjacent to the walls as the peripheral area and the center as the central area **[1-TXT]**.

4.2.1. Software interface dividing the open field box into 9 squares and labeling the peripheral area and the central area. **TXT: Open field box: 50 cm x 50 cm x 50 cm** *Videographer: Please record the screen for this shot.*

4.3. Place the rat in the central area of the open field box **[1-TXT]** and record its movement for 5 minutes using the tracking system **[2]**.

4.3.1. Talent placing a rat in the center of the box. **TXT: Before the experiment, acclimate the rats to the behavioral room for 1 h**

4.3.2. The rat's movement being recorded using the tracking system.

4.4. After testing each rat, clean the open field chamber with 75 percent ethanol to remove residual odor and prevent interference with the behavior of the next rat **[1]**. Enter the total distance of open field activities and the number of entries in the central grid in the open-field test records **[2]**.

4.4.1. Talent spraying and wiping the chamber with ethanol.

4.4.2. Talent entering the data into OFT records.

5. Forced Swimming Test (FST)

Demonstrator: Tongzheng Liu

5.1. Take a transparent plexiglass water cylinder and fill it with water at a temperature of 23 to 25 degrees Celsius. Adjust the water depth according to the animal's weight, ensuring the rat's tail stays a certain distance above the bottom **[1-TXT]**.

5.1.1. Talent pouring water into the plexiglass cylinder. **TXT: Plexiglass water cylinder: 50 cm height, 20 cm diameter**

5.2. After activating the camera and signal acquisition system, slowly place the rats into the water cylinder, maintaining silence throughout the experiment **[1-TXT]**. Observe and record the duration of floating immobility within 300 seconds **[2]**. At the end of the session, immediately remove the rats from the water and dry them **[3]**.

5.2.1. Talent gently placing the rat into the water cylinder. **TXT: Move the rat to the behavioral room 30 min before the experiment for acclimation**

5.2.2. SCREEN: 68198_screenshot_5.2.2.mp4 00:00-00:15.

5.2.3. Talent lifting the rat out and drying it gently with a towel.

5.3. After each session, replace the water in the cylinder to prevent any influence on the next rat **[1]**.

5.3.1. Talent refilling the empty cylinder with clean water.

6. Network Pharmacological Prediction

Demonstrator: Tongzheng Liu

6.1. Access the Traditional Chinese Medicine Systems Pharmacology or TCMSP *(T-C-M-S-P)* database **[1]**, the HERB *(Herb)* database **[2]**, and TCMID *(T-C-M-I-D)* database **[3]**. Use the names of the eight traditional Chinese medicines in Qiangzhifang as keywords to search for active compounds and targets of the herbs **[4]**. Collect target information from TCMSP *(T-C-M-S-P)* and the Swiss target prediction database, setting the filter value to Probability greater than zero **[5]**.

6.1.1. SCREEN: 68198_screenshot_6.1.mp4 00:11-00:17.

6.1.2. SCREEN: 68198_screenshot_6.1.mp4 00:22-00:27.

6.1.3. SCREEN: 68198_screenshot_6.1.mp4 00:32-00:55.

6.1.4. SCREEN: 68198_screenshot_6.1.mp4 01:12-01:28, 01:51-01:54, 02:18-02:22.

6.1.5. SCREEN: 68198_screenshot_6.1.mp4 02:40-03:26.

6.2. Now, navigate to the GeneCards *(Gene-Cards)* database and search for the keyword “depression” **[1]**. After obtaining the gene targets associated with depression, download the electronic spreadsheet, filter out the gene scores that are higher than the average, and compile a list of depression-related targets **[2]**.

6.2.1. SCREEN: 68198_screenshot_6.2.mp4 00:03-00:10.

6.2.2. SCREEN: 68198_screenshot_6.2.mp4 00:11-00:20, 00:46-end.

6.3. Now, access the Jvenn tool and upload the compound targets, followed by the disease target files **[1]**. Plot the overlapping genes between the compound presumed targets and disease targets **[2]**. Then, download the diagram image **[3]**.

6.3.1. SCREEN: 68198_screenshot_6.3.mp4 00:03-00:54.

6.3.2. SCREEN: 68198_screenshot_6.3.mp4 00:57-01:17.

6.3.3. SCREEN: 68198_screenshot_6.3.mp4 01:18-end.

6.4. Finally, access the STRING *(String)* database and paste the overlapping genes, specifically, the Qiangzhifang anti-depression overlapping target list, into the **List of Names** dialog box **[1]**. Select **Homo sapiens** in the Organisms section and click on **SEARCH** followed by **CONTINUE [2]**. Select the **Exports** option from the title bar and download the Protein-protein interaction network summary table in PNG and TSV formats **[3]**.

6.4.1. SCREEN: 68198_screenshot_6.4.mp4 00:06-00:27.

6.4.2. SCREEN: 68198_screenshot_6.4.mp4 00:35-01:02.

6.4.3. SCREEN: 68198_screenshot_6.4.mp4 01:03-end.

Results

7. Results

7.1. In the sucrose preference test, after 28 days **[1]**, the chronic restraint stress group had significantly lower sucrose preference **[2]** than the control **[3]**, while both qiangzhifang- and fluoxetine-treated groups showed significantly higher values compared to the stress group **[4]**.

7.1.1. LAB MEDIA: Figure 2A.

7.1.2. LAB MEDIA: Figure 2A. *Video Editor: Highlight the pink bar in day 28.*

7.1.3. LAB MEDIA: Figure 2A. *Video Editor: Highlight the green bar in day 28*

7.1.4. LAB MEDIA: Figure 2A. *Video Editor: Highlight the grey and yellow bars in day 28.*

7.2. Body weight gain was significantly reduced in the chronic restraint stress group compared to control after 28 days **[1]**, but significantly increased in the qiangzhifang and fluoxetine groups compared to the stress group **[2]**.

7.2.1. LAB MEDIA: Figure 2B. *Video Editor: Highlight the pink and green bars in day 28.*

7.2.2. LAB MEDIA: Figure 2B. *Video Editor: Highlight the pink, yellow, and grey bars in day 28.*

7.3. Open field movement plots showed reduced central area exploration in the stress group **[1]**, while both treatment groups exhibited visibly more central activity **[2]**.

7.3.1. LAB MEDIA: Figure 2C. *Video Editor: Highlight the CRS image.*

7.3.2. LAB MEDIA: Figure 2C. *Video Editor: Highlight the CRS+QZF and CRS+Fluoxetine images.*

7.4. Total distance traveled in the open field test showed no significant differences across all groups **[1]**.

7.4.1. LAB MEDIA: Figure 2D.

7.5. Time in the central area was also significantly reduced in the stress group **[1]**, and significantly increased in both treatment groups **[2]**.

7.5.1. LAB MEDIA: Figure 2E. *Video Editor: Highlight the pink bar.*

7.5.2. LAB MEDIA: Figure 2E. *Video Editor: Highlight the yellow and grey bars.*

7.6. Furthermore, immobility time in the forced swimming test was higher in the stress group **[1]**, and significantly reduced with both treatments **[2].**

7.6.1. LAB MEDIA: Figure 2F. *Video Editor: Highlight the pink bar.*

7.6.2. LAB MEDIA: Figure 2F. *Video Editor: Highlight the yellow and grey bars.*

Pronunciation Guide:
1. Qiangzhifang
Pronunciation link:
https://www.howtopronounce.com/qiangzhifang
IPA: /tʃjɑːŋˈʒɪ.fɑːŋ/
Phonetic Spelling: chyang-zhi-fahng

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2. Pharmacology
Pronunciation link:
https://www.merriam-webster.com/dictionary/pharmacology
IPA: /ˌfɑːr.məˈkɑː.lə.dʒi/
Phonetic Spelling: far-muh-kaw-luh-jee

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3. Fluoxetine
Pronunciation link:
https://www.merriam-webster.com/dictionary/fluoxetine
IPA: /ˈfluː.əkˌsiːˌtiːn/
Phonetic Spelling: floo-uhk-steen

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4. Gavage
Pronunciation link:
https://www.merriam-webster.com/dictionary/gavage
IPA: /gəˈvɑːʒ/
Phonetic Spelling: guh-vahzh

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5. Sucrose
Pronunciation link:
https://www.merriam-webster.com/dictionary/sucrose
IPA: /ˈsuː.kroʊs/
Phonetic Spelling: soo-krohss

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6. Open-Field Test
Pronunciation link:
No confirmed link found
IPA: /ˈoʊ.pən fiːld tɛst/
Phonetic Spelling: oh-puhn feeld test

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7. Plexiglass
Pronunciation link:
https://www.merriam-webster.com/dictionary/plexiglass
IPA: /ˈplɛk.siˌglæs/
Phonetic Spelling: plek-see-glass

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8. Immobility
Pronunciation link:
https://www.merriam-webster.com/dictionary/immobility
IPA: /ˌɪ.moʊˈbɪ.lə.ti/
Phonetic Spelling: ih-moh-bil-ih-tee

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9. Jvenn
Pronunciation link:
https://www.howtopronounce.com/jvenn
IPA: /dʒeɪ.vɛn/
Phonetic Spelling: jay-ven

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10. STRING (database)
Pronunciation link:
https://www.howtopronounce.com/string
IPA: /strɪŋ/
Phonetic Spelling: string

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11. GeneCards
Pronunciation link:
https://www.howtopronounce.com/genecards
IPA: /ˈdʒiːn.kɑːrdz/
Phonetic Spelling: jeen-kardz

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12. TCMSP
Pronunciation link:
https://www.howtopronounce.com/tcmsp
IPA: /tiː-siː-ɛm-ɛs-piː/
Phonetic Spelling: tee-see-em-ess-pee

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13. TCMID
Pronunciation link:
https://www.howtopronounce.com/tcmid
IPA: /tiː-siː-ɛm-aɪ-diː/
Phonetic Spelling: tee-see-em-eye-dee