FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68435
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=20872723

Title: Hypoxia Alters miRNAs Levels Involved in Non-Mendelian Inheritance of Autism Spectrum Disorder in Mice

Authors and Affiliations:
Ecma Güvenilir[1,2], Zeynep Yilmaz Şükranli[2,3], Mai R S Abusalim[1,2], Aslı Okan Oflamaz[4], Züleyha Doğanyiğit[4], Kemal Erdem Başaran[2,5], Mehmet Dolanbay[6], Minoo Rassoulzadegan[2,7,8], Serpil Taheri[2,3*]

[1]Gevher Nesibe Genome and Stem Cell Institute, Erciyes University
[2]Betül Ziya Eren Genome and Stem Cell (GENKÖK) Center, Erciyes University
[3]Department of Medical Biology, Erciyes University
[4]Department of Histology and Embryology, Yozgat Bozok University
[5]Department of Physiology, Erciyes University
[6]Department of Obstetrics and Gynecology, Faculty of Medicine, Erciyes University
[7]University of Nice Sophia Antipolis
[8]Department of Medical Genetics, Erciyes University

Corresponding Authors:
Serpil Taheri (serpiltaheri@hotmail.com)
Email Addresses for All Authors:

Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No.**

NOTE: Authors could not film any of the SCREEN shots due to technical issues. Most of the SC shots have been converted to on-screen text

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **15/07/2025**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare

Current Protocol Length

Number of Steps: 26
Number of Shots: 56

Introduction

NOTE: Authors' names have not been provided

1.1. Enter author name.: We studied how different levels of neonatal hypoxia affect brain development, behavior, and ASD-related molecular changes in mice, emphasizing the severity-dependent effects and need for early intervention..

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.1*

What are the most recent developments in your field of research?

1.2. Enter author name: Recent studies link neonatal hypoxia to autism-related brain changes. Focus is shifting to repurposed drugs targeting neuroinflammation and neuroimmune dysfunction to prevent neuronal damage and long-term cognitive decline.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.8*

What technologies are currently used to advance research in your field?

1.3. Enter author name: We use hypoxia chambers, qPCR, RNA sequencing, confocal imaging, and behavioral tracking to study neonatal hypoxia, with emerging tools like single-cell transcriptomics and CRISPR enhancing precision and insight.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.2*

What are the current experimental challenges?

1.4. Enter author name: A key challenge is translating molecular changes, like miRNA dysregulation, into behavior due to ASD's complexity. Limitations include species differences, lack of sex-specific analysis, and difficulty controlling hypoxia severity.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.18*

What significant findings have you established in your field?

1.5. Enter author name: Our study shows that even mild neonatal hypoxia causes lasting behavioral and molecular changes in mice, with dose-dependent effects and potential miRNA biomarkers, emphasizing early detection and intervention.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.3*

Ethics Title Card

This research has been approved by the Local Animal Experiments Ethics Committee at Erciyes University (HAYDEK)

Protocol

2. Hypoxia Model Establishment in Neonatal Mice

Demonstrator: Kemal Erdem Başaran

2.1. To begin, design the experiment to include four groups made of three hypoxic groups and one sham control group **[1]**. Mate healthy female mice with healthy male mice to generate pregnant female mice **[2]**.

2.1.1. WIDE: Talent setting up four labeled cages representing each experimental group.

2.1.2. Talent placing male and female mice together in mating cages.

2.2. Transfer the pregnant mice to the hypoxia laboratory 72 hours before embryonic day 21.5 and begin monitoring them **[1]**. Place the mothers into hypoxia chambers at the time of delivery **[2]**. Adjust the oxygen levels in each chamber according to the specifications for each group **[3]**.

2.2.1. Talent moving pregnant mice into the hypoxia laboratory and initiating monitoring.

2.2.2. Talent transferring mothers in labor, into hypoxia chambers.

2.2.3. Talent adjusting the oxygen level in the chamber.

~~2.3. Maintain the neonates in hypoxic conditions for five minutes only **[1]**. Then, immediately transfer them to standard housing conditions **[2]**.~~

~~2.3.1. Talent placing neonates into hypoxia chambers.~~

~~2.3.2. Talent transferring neonates to standard housing after five minutes.~~

AUTHOR'S NOTE: step not filmed

~~2.4.~~ Select two neonates from each group to confirm hypoxia-induced damage and determine sex **[1]**. ~~Keep the remaining pups with their mothers in normal housing conditions until the end of the lactation period for further testing **[2]**.~~

2.4.1. Talent selecting two neonates from each group for assessment.

~~2.4.2. Talent returning remaining pups to their mothers in normal housing.~~

AUTHOR'S NOTE: step not filmed

3. Behavioral Assessment in Adult Mice with Induced Neonatal Hypoxia

Demonstrator:

3.1. To perform the novel object recognition test, place a 2-month-old mouse in an arena with two identical objects, allowing it to explore freely **[1]**.

3.1.1. Talent placing mouse in arena with two identical objects.

3.2. ~~During the 10-minute session, observe and record the time the mouse spends with each object and the number of interactions **[1].** Also,~~ Record the number of times the mouse turned toward or interacted with each object **[2].**

~~3.2.1. Talent recording time and interactions with each object.~~
AUTHOR'S NOTE: step not filmed

3.2.2. Shot of the mouse turning or interacting with the object.
AUTHOR'S NOTE: 3.1.1 and 3.2.2 are combined in same clip, i.e. 3.1.1

3.3. The next day, replace one of the familiar objects with a novel object **[1]**. Return the mouse to the arena and allow it to explore for another ten minutes **[2-TXT]**.

3.3.1. Talent replacing one object with a novel object.

3.3.2. Talent returning mouse to arena for second exploration. **TXT: Calculate the DI index**

~~3.4. Record the time spent with the familiar and novel objects and the number of interactions **[1].** Calculate the discrimination index to assess the mouse's memory and learning abilities **[2-TXT].**~~
AUTHOR'S NOTE: step not filmed

~~3.4.1. Talent recording time and interactions with familiar and novel objects.~~

~~3.4.2. SCREEN: The DI index is being calculated. **TXT: Use DI to objectively assess mouse's learning and memory abilities**~~

3.5. To perform the tail suspension test, install the camera to clearly monitor the mice **[1].** ~~Then set up the appropriate video tracking software **[2].**~~

3.5.1. Talent setting up camera.

~~3.5.2. SCREEN: The video tracking software is being seen/installed~~.
AUTHOR'S NOTE: step not filmed

3.6. Prepare the experimental setup to accommodate three mice simultaneously **[1]**. Use 25-centimeter-high cardboard panels with 20-centimeter intervals to isolate the mice **[2]**.

3.6.1. Shot of the setup.

3.6.2. Talent placing cardboard panels to isolate mice.

3.7. Then cut 12-centimeter-long strips of tape **[1]** and attach them to the end of each mouse's tail, positioning the tape 2 centimeters from the tip to suspend the mouse safely **[2]**.

3.7.1. Talent cutting 12 cm strips of tape.

3.7.2. Talent attaching tape to mouse tails for suspension.

3.8. Record the mice for six minutes while suspended **[1]**. After the test, stop the recording, remove the mice, and return them to their housing cages **[2-TXT]**. ~~Analyze the videos to determine the duration of active movement and immobility **[3].**~~

3.8.1. Shot of suspended mice.

3.8.2. Talent removing mice and returning them to cages. **TXT: Analyse the videos to determine the duration of active movement and immobility**

~~3.8.3. SCREEN: Video of the suspended mouse is being seen.~~

3.9. For the marble burying test, fill an empty cage with a 5-centimeter layer of corn cob bedding **[1]**. Arrange 20 marbles in five rows of four on the bedding surface **[2]**.

3.9.1. Talent filling cage with corn cob bedding.

3.9.2. Talent arranging marbles on bedding surface.
AUTHOR'S NOTE: 3.9.1-3.9.2 are shot together

3.10. Place the subject mouse in a corner of the cage, allowing free access to the entire area **[1]**. Allow the mouse to explore for 20 minutes, during which it may bury marbles **[2]**.

3.10.1. Talent placing mouse in cage corner.

3.10.2. Shot of the mouse exploring the cage and burying marbles.

3.11. After 20 minutes, remove the mouse **[1]** and count the number of marbles buried under the bedding **[2-TXT]**. ~~Record the total number of marbles buried to assess anxiety-related behaviors and compulsive tendencies **[3].**~~

3.11.1. Talent removing mouse from the setup.

3.11.2. Talent counting buried marbles. **TXT: Record toral number of buried marbles to assess anxiety and compulsive tendencies**

~~3.11.3. SCREEN: Display recorded number of marbles buried for behavioral assessment.~~

3.12. Next, perform the social interaction test. Install the camera to clearly monitor the mice **[1]** and connect it to the computer with appropriate video tracking software **[2]**.

3.12.1. Talent setting up camera.

3.12.2. Talent connecting the camera to the computer with video tracking software.

3.13. Set up a rectangular box with two walls containing two doors to create three chambers **[1]**. Place a cage containing a mouse that is familiar with the experimental setup in one chamber **[2]** and another cage with a mouse that had no previous experience, in another chamber **[3]**.

3.13.1. Shot of the rectangular box with 3 chambers.

3.13.2. Talent placing labeled cage with familiar mouse in one chamber.

3.13.3. Talent placing labeled cage with unfamiliar mouse in 2nd chamber.

3.14. Start recording and place the subject mouse in the middle compartment, allowing it to explore for five minutes **[1]**. Return the mouse to its housing cage after the session **[2-TXT]**.

3.14.1. Talent recording subject mouse exploring for five minutes.

3.14.2. Talent removing subject mouse after session. **TXT: Analyse the interaction time and compare with the sham control**

~~3.15. Use the social interaction test software to analyze the time spent interacting with each mouse and compare results with the sham control group **[1]**.~~

~~3.15.1. SCREEN: The interaction time is being analyzed and compared with sham control group.~~
AUTHOR'S NOTE: step not filmed

3.16. To perform the open field test, position the camera and connect it to the computer **[1]**. Then set up the test arena and divide it with imaginary lines to create 16 squares **[2]**.

3.16.1. Talent setting up camera and video tracking software.

3.16.2. Talent arranging test arena with 16 imaginary squares.

3.17. Start recording and carefully place the mouse in the center of the arena, observing its behavior for five minutes **[1]**. After the session, stop the recording, remove the mouse, and return it to its housing cage **[2]**.

3.17.1. Talent placing mouse in center and recording behavior.

3.17.2. Talent removing mouse after five minutes.

3.18. Use the tracking software to measure behaviors to assess anxiety levels, locomotor activity, exploratory behavior, and emotional responses **[1]**.

3.18.1. ~~SCREEN: Display analysis of recorded behaviors for assessment.~~
~~**AND**~~
TEXT ON PLAIN BACKGROUND:

Measure the following behaviors:

Walking
Climbing
Escape Attempts
Grooming Immobility
Total Crossings
Defecation
Urination

3.19. To perform the Morris water maze test, prepare a water maze in a stable position within a behavioral laboratory **[1-TXT]**. Hang distinct visual cues at the center of four imaginary quadrants of the maze wall, approximately 20 centimeters from the base **[2]**.

3.19.1. Talent setting up water maze in behavioral laboratory. **TXT: Maze dimensions: 120 cm (diameter), 60 cm (depth); Maintain lab at 23 ± 1 °C**

3.19.2. Talent hanging visual cues in each quadrant.

3.20. Next, position a platform measuring 17 centimeters in length and 10 centimeters in diameter about 20 centimeters from the wall **[1]**. Fill the maze with water at a temperature between 21 and 26 degrees Celsius to a level 1 centimeter below the platform **[2].** Stain it with a black multi-surface acrylic dye **[3]**.

3.20.1. Talent positioning platform in maze.

3.20.2. Talent filling maze with appropriately tempered water.

3.20.3. Talent adding black dye to the water.

3.21. Place the camera to capture the full maze **[1].** Connect it to the Morris water maze software on the computer **[2]**.

3.21.1. Talent setting up camera.

3.21.2. Talent connecting to software.

3.22. Begin recording and place the subject mouse in a different quadrant for the first four days, with its face directed toward the tank wall **[1]**. Assign the platform location in the day five test schedule **[2]**. On the fifth day, remove the platform **[3]** and carefully place the mice in a square of the maze as demonstrated **[4]**.

3.22.1. Talent placing mouse in different quadrant each day and recording.

3.22.2. Talent assigning platform location for day five.

3.22.3. Talent removing platform on day five.

3.22.4. Talent placing the mice in a maze square.

Results

4. Results

4.1. In the novel object recognition test, mice exposed to 8%, 10%, and 12% hypoxic oxygen conditions during birth showed significantly greater exploration of the novel object **[1]**.

4.1.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the blue, red, and green bars*

4.2. Total distance traveled was significantly reduced in the 8%, 10%, and 12% oxygen groups compared to the 21% oxygen group **[1]** with a corresponding significant reduction in velocity **[2]**.

4.2.1. LAB MEDIA: Figure 5D. *Video editor: Emphasize the blue, red, green bars*

4.2.2. LAB MEDIA: Figure 5F. *Video editor: Emphasize the blue, red, green bars*

4.3. Discrimination index scores were significantly higher in the 10% and 12% oxygen groups, indicating increased novelty preference **[1]**. The percentage of novel object discovery was significantly reduced in the 10% and 12% oxygen groups **[2]**.

4.3.1. LAB MEDIA: Figure 5H. *Video editor: Highlight the red and green bars*

4.3.2. LAB MEDIA: Figure 5J. *Video editor: Highlight the red and green bars*

4.4. Marble burying behavior was significantly reduced in the 8%, 10%, and 12% oxygen groups, with the most pronounced decrease observed in the 10% oxygen group **[1]**.

4.4.1. LAB MEDIA: Figure 7A. *Video editor: Highlight the blue, red, and green bars*

4.5. In the SIT *(S-I-T)* , male mice of the 21% group showed a significantly greater preference for the cage **[1]**. Distance traveled significantly decreased in the 10% and 12% oxygen groups **[2]**. Only the female mice of the 21% group traveled more than males **[3]**.

4.5.1. LAB MEDIA: Figure 8B. *Video editor: Highlight the purple male bar*

4.5.2. LAB MEDIA: Figure 8C. *Video editor: Highlight the red and green bars for 10% and 12% oxygen groups*

4.5.3. LAB MEDIA: Figure 8D. *Video editor: Emphasize the purple female bar*

4.6. Movement speed during the was significantly lower in the 10% and 12% oxygen groups **[1]**. Female mice in the 21% oxygen group moved significantly faster than males **[2]**.

4.6.1. LAB MEDIA: Figure 8E. *Video editor: Highlight the blue and red bars for 10% and 12% oxygen.*

4.6.2. LAB MEDIA: Figure 8F. *Video editor: Please highlight the purple female bar*

4.7. In the Morris Water Maze test, interest in the platform decreased from day 1 to day 5 in all groups, indicating memory decline **[1]**. In the open field test, all groups spent more time in the peripheral area than in the central area, suggesting anxiety-related behavior **[2]**.

4.7.1. LAB MEDIA: Figure 9B. *Video editor: Sequentially highlight the bars from 1st day to 5th day*

4.7.2. LAB MEDIA: Figure 10A. *Video editor: Please Highlight the PA bars*

4.8. Significant sex-based differences were observed across all groups, though no differences were found between sexes within individual groups **[1]**. All oxygen groups showed increased total movements **[2]**.

4.8.1. LAB MEDIA: Figure 10B.

4.8.2. LAB MEDIA: Figure 10E.

Pronunciation Guide:

miRNAs
Pronunciation link: https://www.merriam-webster.com/dictionary/miRNA Merriam-Webster
IPA: /ˌmaɪ.kroʊ.rɪˈen.eɪz/
Phonetic Spelling: my-kroh-ree-EN-ayz

Hypoxia
Pronunciation link: https://www.merriam-webster.com/dictionary/hypoxia
IPA: /haɪˈpɒk.si.ə/ (US: /haɪˈpɑːk.si.ə/)
Phonetic Spelling: hy-POK-see-uh

Non-Mendelian
Pronunciation link: https://www.synonyms.com/pronounce/non-mendelian+inheritance synonyms.com+1
IPA: /nɒn-ˌmɛnˈdiːliən/ (US: /nɑn-ˌmɛnˈdiːliən/)
Phonetic Spelling: non-MEN-dee-lee-ən

Inheritance
Pronunciation link: https://www.merriam-webster.com/dictionary/inheritance
IPA: /ɪnˈhɛr·ɪ·təns/
Phonetic Spelling: in-HER-i-tans

Autism
Pronunciation link: https://www.merriam-webster.com/dictionary/autism
IPA: /ˈɔː.tɪz.əm/ (US: /ˈɔː.tɪz.əm/ or /ˈɑː.tɪz.əm/)
Phonetic Spelling: AW-tiz-um

Syndrome
Pronunciation link: https://www.merriam-webster.com/dictionary/syndrome
IPA: /ˈsɪnˌdroʊm/
Phonetic Spelling: SIN-drome

Neonatal
Pronunciation link: https://www.merriam-webster.com/dictionary/neonatal
IPA: /ˌniːoʊˈneɪtəl/
Phonetic Spelling: nee-oh-NAY-tuhl

Behavior
Pronunciation link: https://www.merriam-webster.com/dictionary/behavior
IPA: /bɪˈheɪ·vjər/ (US)
Phonetic Spelling: bi-HAY-vyur

Transcriptomics
Pronunciation link: No confirmed link found
IPA: /ˌtræns.krɪpˈtoʊ.mɪks/
Phonetic Spelling: trans-krip-TOH-miks

Confocal
Pronunciation link: https://www.merriam-webster.com/dictionary/confocal
IPA: /kənˈfoʊkəl/
Phonetic Spelling: kun-FOH-kul

Behavioural (British spelling) / Behavioral
Pronunciation link: https://www.merriam-webster.com/dictionary/behavioral
IPA: /bɪˈheɪ·vjʊr·əl/ (US: /bɪˈheɪ·vjər·əl/)
Phonetic Spelling: bi-HAY-vyuh-rul
qPCR (quantitative Polymerase Chain Reaction)
Pronunciation link for "qPCR": No dedicated entry found
IPA: /ˌkjuːˌpiːˌsiːˈɑːr/
Phonetic Spelling: cue-pee-cee-AR