FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 66311
Scriptwriter Name: Nilesh Kolhe
Project Page Link: https://review.jove.com/account/file-uploader?src=20224903

Title: Measuring Associative Learning in Chemotaxis of the Nematode *Caenorhabditis elegans*

Authors and Affiliations: Daniel-Cosmin Marcu*, Ephraim Immanuel Berthold*, Karl Emanuel Busch

Institute for Mind Brain and Behavior, Faculty of Medicine, HMU Health and Medical University Potsdam

*These authors contributed equally

Corresponding Authors:
Karl Emanuel Busch emanuel.busch@hmu-potsdam.de

Email Addresses for All Authors:
emanuel.busch@hmu-potsdam.de
daniel-cosmin.marcu@hmu-potsdam.de
Ephraimberthold@outlook.de

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? **Yes, all done**

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**

Current Protocol Length
Number of Steps: 24
Number of Shots: 50 (7 SCOPE, 2 TEXT on PLAIN BACKGROUND)

Introduction

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

1.1. **Karl Emanuel Busch:** One of the most fascinating questions in neuroscience is how learning and memory emerge from the plasticity of neurons. Both plasticity and learning decline with age, but it is largely unclear why. We investigate the mechanisms responsible for this decline in the nematode model organism *Caenorhabditis elegans*.

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

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

1.2. **Karl Emanuel Busch:** It has become clear that nearly every behavior of *Caenorhabditis elegans* depends on experience and context and that they share mechanisms of associative and non-associative learning with other animals. Remarkably, key determinants of *Caenorhabditis elegans* learning, such as neural activity or insulin signaling, are also implicated in regulating the aging processes of the brain and even the entire organism.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.1.2*

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

1.3. **Daniel-Cosmin Marcu:** The basic technologies used in studying chemotaxis are divided Petri dishes and stereomicroscopes. We also use cameras with tracking software and custom-built behavior chambers for trajectory tracking and calcium imaging with microfluidics to visualize the physiological mechanisms behind the behaviors we observe on the Petri dish.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.4.3*

What research gap are you addressing with your protocol?

1.4. **Ephraim Berthold:** Currently, assays for *Caenorhabditis elegans* salt chemotaxis use either gradient plates or quadrant plates. Gradient plate assays can be demanding to perform because the salt gradient changes over time, and quadrant plates only allow to compare the response of worms to two different concentrations of sodium chloride.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.11.1*

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

1.5. **Karl Emanuel Busch:** We want to understand why the ability to learn, which depends on neural plasticity, declines with age. We aim to use the chemotaxis assay to test whether specific signaling pathways are responsible for the decline. Based on this, we aim to develop treatments targeting age-dependent cognitive decline.

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.

Testimonial Questions (OPTIONAL):

What motivated you to choose JoVE for publishing your research?

1.6. **Karl Emanuel Busch:** The use of explanatory videos is a real advantage to better reproduce and implement specific experimental assays, especially to study animal behaviour. To perform a particular procedure, the devil is often in the detail, where certain conditions may just not be mentioned in the write-up of a standard publication.

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

Protocol

NOTE: LAB MEDIA/SCREEN/SCOPE timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sulakshana Karkala) for queries regarding lab media.

2. *Caenorhabditis elegans* Maintenance and Associative Learning Test Preparation

Demonstrator: Ephraim Berthold

2.1. To begin, obtain nematode growth medium agar plates fed with live *Escherichia coli (Esh-uh-RIK-ee-uh KOH-lai)* OP50 *(O-P-fifty)* **[1].** Add *Caenorhabditis elegans (See-nuh-RAB-dih-tis EL-ih-ganz)* N2 on the plate **[2]** and maintain overnight at 20 degrees Celsius upside down to minimize moisture loss **[3]**.

2.1.1. WIDE: Establishing shot of talent bringing prepared NGM GP to the workbench or LAF workstation.

2.1.2. SCOPE: 2-1-2 Take 1.mp4 00:11-00:19, 00:33-00:47

2.1.3. Talent placing the plate upside down inside a 20 degrees Celsius incubator.

2.2. The next day, transfer 30 young adult *Caenorhabditis elegans* onto the nematode growth plate **[1]**. Allow animals to lay eggs for approximately 8 hours at 20 degrees Celsius **[2]**.

2.2.1. Talent transferring *C. elegans* onto a growth plate.

2.2.2. Talent placing the plate in an incubator.

2.3. After the timed egg-laying, remove all adult worms from the plate **[1]**. Culture the plate upside down at 20 degrees Celsius for 3 days **[2]**.

2.3.1. SCOPE: 2-3-1.mp4 00:35-00:45

2.3.2. Shot of the plates being inverted and placed in the incubator.

2.4. For the assay, seed half of the batch of the desired groups of conditioning plates with 200 microliters of OP50 per plate **[1-TXT].**

2.4.1. Talent pipetting 200 microliters of OP50 onto plate. **TXT: Conditioning plates: 0 mM NaCl, 50 mM NaCl, and 100 mM NaCl**

2.5. Allow the seeded plates to dry overnight at 20 degrees Celsius in an incubator alongside the unseeded plates **[1]**.

2.5.1. Talent placing the seeded and unseeded plates in an incubator.

2.6. Prepare desired concentration of 1 liter sodium chloride solution in a glass bottle **[1]**.

2.6.1. Talent adding ingredients in 1 L glass bottle containing D/W with stirrer and other two 1 L bottles placed next to it.

AND

TEXT on PLAIN BACKGROUND:

NaCl (mM)	NaCl (gm)	Peptone (gm)	Agar (gm)	D/W (mL)
0	0	2.5	17	975
50	3	2.5	17	975
100	6	2.5	17	975

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

2.7. Autoclave the bottles for 15 minutes at 121 degrees Celsius **[1]**. Then, let the bottles cool for 30 minutes at 55 degrees Celsius in a water bath **[2]**.

2.7.1. Talent placing the bottles in an autoclave.

2.7.2. Talent transferring the bottles to a water bath.

2.8. Meanwhile, label the bottom of the Y-Petri dish section with the corresponding sodium chloride concentration **[1]** and draw a 5-millimeter radius circle around the plate center where the walls meet **[2]**.

2.8.1. Talent labeling the bottom of the Y-Petri dish.

2.8.2. Talent drawing the circle with precision in the center of Y-Petri dish.

2.9. Place each bottle, one at a time, on a heated magnetic stirrer **[1]**. Add 25 milliliters of 1 molar potassium phosphate solution at pH 6 to each bottle **[2]**. Mix thoroughly for 2 minutes **[3]**. Then, sequentially add calcium chloride, cholesterol, and magnesium sulfate while stirring **[4-TXT]**.

2.9.1. Talent placing a bottle on the heated magnetic stirrer.

2.9.2. Talent adding 25 milliliters of potassium phosphate solution to the bottle.

2.9.3. Shot of the bottle on a magnetic stirrer with visible mixing/starting the stirring.

2.9.4. Talent adding calcium chloride, cholesterol in ethanol, and magnesium sulfate solutions to the bottle while stirring being on. **TXT: 1 mL $CaCl_2$ (1 M), 1 mL cholesterol in ethanol (5 mg/mL), 1 mL $MgSO_4$ (1 M)**

2.10. Next, aliquot the 0-millimolar medium into one compartment of a Y-Petri dish until it reaches the top of the internal wall and let the medium solidify for approximately 5 minutes **[1]**.

2.10.1. Talent aliquoting the 0 millimolar medium into the first compartment of the

Y-Petri dish.

2.11. Aliquot the 100-millimolar medium into the second compartment of the Y-Petri dish until it touches the solidified 0-millimolar section adjacent to it **[1]**. Then, use a sterile 10-microliter pipette tip to break the surface tension along the Y-wall and connect it to the 0-millimolar sodium chloride section. Let the medium solidify for 5 minutes **[2]**.

2.11.1. Talent aliquoting the 100-millimolar medium into the second compartment of the Y-Petri dish. **TXT: To prevent spillover, fill the 100 mM section to the top of the internal Y-wall**

2.11.2. Talent breaking the surface tension with a 10-microliter pipette tip and connecting the medium to the 0-millimolar NaCl section.

2.12. Aliquot the 50-millimolar medium into the remaining compartment of the Y-Petri dish until it touches the solidified 0-millimolar and 100-millimolar sections **[1]**. Then, use a sterile 10-microliter pipette tip to break the surface tension along the Y-wall and connect the 50-millimolar sodium chloride section to the other two sections. **[2]**. After letting the medium solidify for 5 minutes, store the plates overnight at 20 degrees Celsius **[3]**.

2.12.1. Talent aliquoting the 50-millimolar medium into the last compartment of the Y-Petri dish.
Videographer's Note: Use shot labeled "Real clapperboard 2.12.0"

Added shot: Talent breaking the surface tension with a 10-microliter pipette tip
Videographer's Note: Use shot labeled "Real clapperboard 2.12.0,5"

2.12.2. Talent placing the solidified dishes in an incubator.
Videographer's Note: Use shot labeled "Real clapperboard 2.12.1"

3. Preparation and Conditioning of *Caenorhabditis elegans* for Chemotaxis Assay Index Calculation

Demonstrator: Daniel-Cosmin Marcu

3.1. To begin, pipette 1 milliliter of sterile M9 buffer onto a growth plate containing *Caenorhabditis elegans* **[1]**. Swirl the plate gently to dislodge the worms from the OP50 bacterial lawn and the agar, forming a suspension **[2]**.

3.1.1. WIDE: Talent pipetting M9 buffer onto a growth plate.

3.1.2. Talent gently swirling the plate.

3.2. Tilt the plate to collect the worm suspension in M9 buffer using a 1-milliliter plastic pipette tip with the top part cut off **[1]**. Transfer the suspension to a 1.5-milliliter microcentrifuge tube **[2]**. Allow the worms to settle at the bottom of the microcentrifuge tubes for approximately 60 seconds, forming a pellet **[3]**.

3.2.1. Talent tilting the plate and collecting the worm suspension using a pipette

tip with top part cut off.

3.2.2. The suspension being transferred to a microcentrifuge tube.

3.2.3. SCOPE: 3-2-3.mp4 Take 2 00:08-00:12, 01:26-01:40,

3.3. Then, remove the supernatant using a 1-milliliter pipette without disturbing the pellet **[1]**. Wash the pellet with 1 milliliter of sterile M9 buffer twice to remove residual OP50 **[2-TXT]**. Resuspend the pellet in 300 microliters of M9 buffer after the final wash **[3]**.

3.3.1. Talent removing supernatant from the microcentrifuge tube with a pipette.

3.3.2. Talent adding 1 milliliter of M9 buffer into the tube. **TXT: Let the worms settle for about 60 s between washes**

3.3.3. Talent resuspending the pellet in 300 microliters of M9 buffer.

3.4. Swirl the suspension for even distribution of worms **[1]** and slowly transfer 50 microliters of worm suspension, containing approximately 200 to 300 worms, to each conditioning plate **[2-TXT]**. For plates seeded with OP50, deposit the worm droplet away from the bacterial food lawn and let it get absorbed into the agar **[3]**. Keep the plates upside down in an incubator at 20 degrees Celsius for 4 hours **[4]**.

3.4.1. Talent swirling the worm suspension tube.

3.4.2. Talent pipetting worm suspension onto a conditioning plate. **TXT: CP0-, CP0+, CP50-, CP50+, CP100- and CP100+**

3.4.3. SCOPE: 3-4-3.mp4. 00:06-00:33

3.4.4. Talent placing the plates in an incubator.

3.5. After conditioning, pipette 1 milliliter of sterile M9 buffer onto a conditioning plate **[1]** and swirl it gently to dislodge the worms, forming a suspension **[2]**. Then, tilt the plate to collect the worm suspension using a 1-milliliter plastic pipette tip **[3]** and transfer it to a 1.5-milliliter microcentrifuge tube **[4]**.

3.5.1. Talent pipetting M9 buffer onto the conditioning plate.

3.5.2. SCOPE: 3-5-2.mp4. 00:04-00:20

3.5.3. Talent tilting the plate and collecting the worm suspension.

3.5.4. Suspension being transferred into a microcentrifuge tube.

3.6. In 60 seconds, the worms settle down and form a pellet **[1]**. After removing the supernatant, wash the worms two times with 1 milliliter of sterile M9 buffer **[2-TXT]**.

3.6.1. SCOPE: 3-6-1.mp4. 00:12-00:17, 01:40-01:47

3.6.2. Talent removing M9 buffer from the microcentrifuge tube containing worm

pellet. **TXT: Let the worms settle for ~60 s between two washes**

3.7. Then, fill the microcentrifuge tube with M9 buffer up to the 1-milliliter mark **[1]**. Once the worm pellet forms, remove 960 microliters of the supernatant without disturbing the pellet **[2]**.

3.7.1. Talent filling the tube to the 1 milliliter mark with M9 buffer.

3.7.2. Talent removing supernatant from the tube containing pellet.

3.8. Now, swirl or shake the tube gently to resuspend the worms **[1]**. Transfer 20 microliters of this suspension, containing approximately 100 to 150 worms, to the center of an assay plate where the Y-wall segments meet **[2-TXT]**.

3.8.1. Talent gently swirling the tube to resuspend the worms.

3.8.2. SCOPE: 3-8-2.mp4. 00:00-00:24
TXT: Assay plates: 0 mM NaCl, 50 mM NaCl, and 100 mM NaCl

3.9. Let the worms roam for 30 minutes at 20 degrees Celsius **[1]**. Then, count the number of worms in each section of the assay plate and count them again after 30 minutes **[2-TXT]**.

3.9.1. Talent placing the dish in the incubator.

3.9.2. SCOPE: 3-9-2.mp4. 00:08-00:30
TXT: Exclude worms on the section borders or within 5 mm of the center of the plate.
NOTE: Author planned to provide new 3.9.2 but hasn't provided it in 12 days. Please use videographer's footage here

3.10. Calculate the chemotaxis index per plate per time point using the specified formula **[1,2]**.

3.10.1. TEXT ON PLAIN BACKGROUND:

$$CI = \frac{N_{worms\ in\ 100\ mM\ NaCl\ section\ of\ AP} - N_{worms\ in\ 0\ mM\ NaCl\ section\ of\ AP}}{N_{non-excluded\ worms\ in\ all\ sections\ of\ AP}}$$

3.10.2. Talent performing calculations on a computer with a screen visible in the frame. *Video Editor: Please split screen to show 3.10.1 and 3.10.2 side by side*

Results

4. Representative Results

4.1. Wild-type *Caenorhabditis elegans* behavior was significantly affected by 4-hour conditioning on plates with different sodium chloride levels, with or without food **[1]**.

4.1.1. LAB MEDIA: Figure 2

4.2. After 30 minutes on the assay plate, worms conditioned with high sodium chloride concentrations and food **[1]**, as well as those conditioned with no sodium chloride and no food **[2]**, showed a positive chemotaxis index, indicating a preference for higher sodium chloride concentrations **[3]**.

4.2.1. LAB MEDIA: Figure 2 *Video Editor: Please emphasize 'black colored bars (30 min)' from CP100+*

4.2.2. LAB MEDIA: Figure 2 *Video Editor: Please emphasize 'black colored bars (30 min)' from CP0- points*

4.2.3. LAB MEDIA: Figure 2 *Video Editor: Please emphasize 'black colored bars (30 min)' from CP100+ and CP0- points*

4.3. Conversely, worms conditioned with high sodium chloride concentrations and no food **[1]**, medium sodium chloride concentrations and no food **[2]**, and no sodium chloride with food **[3]** showed a negative chemotaxis index, indicating a preference for lower sodium chloride concentrations **[4]**.

4.3.1. LAB MEDIA: Figure 2 *Video Editor: Please emphasize 'black colored bars (30 min)' of CP100-*

4.3.2. LAB MEDIA: Figure 2 *Video Editor: Please emphasize 'black colored bars (30 min)' of CP50- points*

4.3.3. LAB MEDIA: Figure 2 *Video Editor: Please emphasize 'black colored bars (30 min)' of CP0+ points*

4.3.4. LAB MEDIA: Figure 2 *Video Editor: Please emphasize 'black colored bars (30 min)' of CP100-, CP50-, and CP0+ points*

4.4. The sodium chloride-food association persisted after 60 minutes, with the chemotaxis index often changing in worms conditioned with food, indicating dynamic associative learning as animals update this association while roaming and searching for food **[1]**.

4.4.1. LAB MEDIA: Figure 2 *Video Editor: Please emphasize 'grey colored bars (60 min)' from CP100+ and CP0+ points*

Pronunciation Guide:

1. **Caenorhabditis elegans**
   Pronunciation link: https://www.howtopronounce.com/caenorhabditis-elegans How To Pronounce
   IPA: /ˌsiːnəˈræbdɪtɪs əˈlɛgænz/
   Phonetic Spelling: see-nuh-RAB-di-tis eh-LEG-anz
2. **chemotaxis**
   Pronunciation link: https://www.merriam-webster.com/dictionary/chemotaxis
   IPA: /ˌkiːmoʊˈtæksɪs/
   Phonetic Spelling: kee-moh-TAK-sis
3. **nematode**
   Pronunciation link: https://www.merriam-webster.com/dictionary/nematode
   IPA: /ˈnɛm.əˌtoʊd/
   Phonetic Spelling: NEM-uh-tohd
4. **associative**
   Pronunciation link: https://www.merriam-webster.com/dictionary/associative
   IPA: /əˈsoʊsiətɪv/
   Phonetic Spelling: uh-SOH-shee-uh-tiv
5. **plasticity**
   Pronunciation link: https://www.merriam-webster.com/dictionary/plasticity
   IPA: /pləˈstɪsɪti/
   Phonetic Spelling: plus-TISS-ih-tee
6. **stereomicroscope**
   Pronunciation link: https://www.merriam-webster.com/dictionary/stereomicroscope
   IPA: /ˌstɪrioʊˈmaɪskəroʊp/
   Phonetic Spelling: STARE-ee-oh-MY-skrohpe
7. **microfluidics**
   Pronunciation link: no confirmed link found
   IPA: /ˌmaɪkroʊfluːˈɪdɪks/
   Phonetic Spelling: MY-kroh-FLOO-id-iks
8. **trajectory**
   Pronunciation link: https://www.merriam-webster.com/dictionary/trajectory
   IPA: /trəˈdʒɛktəri/
   Phonetic Spelling: truh-JEK-tuh-ree
9. **calcium chloride**
   - **calcium**
     Pronunciation link: https://www.merriam-webster.com/dictionary/calcium
     IPA: /ˈkælsiəm/
     Phonetic Spelling: KAL-see-um
   - **chloride**
     Pronunciation link: https://www.merriam-webster.com/dictionary/chloride
     IPA: /ˈklɔːraɪd/
     Phonetic Spelling: KLOR-ide
10. **magnesium sulfate**

- **magnesium**
  Pronunciation link: https://www.merriam-webster.com/dictionary/magnesium
  IPA: /mægˈniːziəm/
  Phonetic Spelling: mag-NEE-zee-um
- **sulfate**
  Pronunciation link: https://www.merriam-webster.com/dictionary/sulfate
  IPA: /ˈsʌlfeɪt/
  Phonetic Spelling: SUL-fayt

11. **autoclave**
    Pronunciation link: https://www.merriam-webster.com/dictionary/autoclave
    IPA: /ˈɔːtəkleɪv/
    Phonetic Spelling: AW-tuh-klayv
12. **micrometer**
    Pronunciation link: https://www.merriam-webster.com/dictionary/micrometer
    IPA: /ˈmaɪkrəˌmiːtər/
    Phonetic Spelling: MY-kruh-mee-ter
13. **incubator**
    Pronunciation link: https://www.merriam-webster.com/dictionary/incubator
    IPA: /ˈɪŋkjəˌbeɪtər/
    Phonetic Spelling: ING-kyuh-bay-tər