

Submission ID #: 69788

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21285388>

Title: Lumbar Intrathecal Injection of SOD1-ASOs for Precise CNS Targeting and Predictive Efficacy in Human SOD1-G93A ALS Mice

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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. Interview statements: Which interview statement filming option is the most appropriate for your group? **Please select one.**



Interview Statements are read by JoVE's voiceover talent.

Current Protocol Length

Number of Steps: 05

Number of Shots: 08

Introduction

INTRODUCTION:

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

- 1.1. This work focuses on freehand lumbar intrathecal injections to deliver therapeutics directly into the cerebrospinal fluid of mice.

1.1.1. B.roll: 2.2.1

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

- 1.2. This minimally invasive, rapid technique offers superior translational relevance compared with more invasive approaches such as intracerebroventricular injections.

1.2.1. B.roll: 2.4.1

CONCLUSION:

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

- 1.3. The primary procedural challenge is accurately identifying the anatomical entry point to access the lumbar cistern.

1.3.1. B.roll: 2.6.1

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

- 1.4. Future studies may compare the cell type-specific distribution or effects of injected therapeutics between intrathecal delivery and other intra-CSF administration methods.

1.4.1. B.roll: 3.2.1

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Biogen

Protocol

2. Direct Intrathecal Injection of Antisense Oligonucleotides into the Mouse

Demonstrator: Twinkle Chowdhury

2.1. To begin, apply ophthalmic lubricant to both eyes of the mouse immediately after anesthesia induction to prevent corneal desiccation [1-TXT]. Then position the mouse in ventral recumbency on the heating pad to prevent hypothermia, minimize physiological stress, and support normal cardiovascular and respiratory functions throughout the procedure [2].

2.1.1. LAB MEDIA: A)Mouse_Preparation_1 IMG.MOV 00:00–00:10 **TXT: Anesthesia: 2 - 2.5% Isoflurane**

2.1.2. LAB MEDIA: A)Mouse_Preparation_1 IMG.MOV 00:11–00:20

2.2. Administer buprenorphine extended-release injectable suspension at 3.25 milligrams per kilogram subcutaneously using a sterile syringe with a 25-gauge needle and inject 0.05 milliliters per 20 grams of body weight prior to the procedure [1].

2.2.1. LAB MEDIA: A)Mouse_Preparation_1 IMG.MOV 00:20–00:35

2.3. After shaving the lumbar region from mid-flank to pelvic girdle, disinfect the area using alternating swabs of povidone-iodine [1] and isopropyl alcohol. Repeat three times and allow the site to air-dry completely [2].

2.3.1. LAB MEDIA: B)Mouse_Preparation_2 IMG.MOV 00:08–00:14

2.3.2. LAB MEDIA: B)Mouse_Preparation_2 IMG.MOV 00:15–00:25

2.4. Now, stabilize the pelvic girdle by gently grasping the pelvic girdle between the thumb and forefinger of one hand and palpating the dorsal midline with the other hand to locate the target intervertebral space between L4 to L5 or L5 to L6 [1].

2.4.1. LAB MEDIA: C)Mouse IT injection Video IMG.MOV 00:00–00:10

2.5. Align the antisense oligonucleotide-containing sterile 0.3 milliliter insulin syringe fitted with a 29-gauge, ½-inch needle perpendicular to the dorsal skin surface or at a 70 to 80 degree angle, with the bevel oriented cranially [1]. Advance the needle until slight

resistance is encountered as it penetrates the dura mater, then continue into the intrathecal space to inject the solution. Note the tail flick reflex, which indicates successful intrathecal entry [2].

2.5.1. LAB MEDIA: C)Mouse IT injection Video IMG.MOV 00:11–00:25

2.5.2. LAB MEDIA: C)Mouse IT injection Video IMG.MOV 00:26–00:31

Results

3. Results

3.1. Evans blue dye injected intrathecally for foundational validation experiments at 20, 30 microliters, and 50 microliters volumes showed consistent dorsal-to-ventral spinal cord staining in all mice, with higher volumes yielding more intense rostrocaudal dye saturation [1].

3.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight the increasing darkness of blue staining from the 20 , 30 and the 50 microliter Evans blue injection spinal cords.*

3.2. Mice injected with sterile saline instead of Evans blue dye showed no spinal cord coloration or background staining [1].

3.2.1. LAB MEDIA: Figure 1. *Video editor: Show the spinal cord from the control (saline)*

3.3. At 5 weeks post-treatment, compound muscle action potential amplitude was significantly higher in mice treated with SOD1-targeting antisense oligonucleotide via intrathecal injection [1] or intracerebroventricular injection [2], compared to mice treated with inactive antisense oligonucleotide [3]

3.3.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the green triangle group labelled "SOD1-ASO 100 µg; IT" with visibly higher CMAP amplitude.*

3.3.2. LAB MEDIA: Figure 2A. *Video editor: Highlight the blue circle group labelled "SOD1-ASO 100 µg; ICV" with similarly elevated CMAP amplitude.*

3.3.3. LAB MEDIA: Figure 2A. *Video editor: Highlight the red square group labelled "Inactive ASO; IT" with the lowest CMAP amplitude.*

3.4. Serum levels of phosphorylated neurofilament heavy chain at 5 weeks post-injection were significantly reduced in both intrathecal [1] and intracerebroventricular SOD1-antisense oligonucleotide-treated mice [2] compared to inactive ASO controls [3].

3.4.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the green triangle group labelled "SOD1-ASO 100 µg; IT" with low pNFH levels.*

3.4.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the blue circle group labelled "SOD1-ASO 100 µg; ICV" with similar low pNFH levels.*

3.4.3. LAB MEDIA: Figure 2B. *Video editor: Highlight the red square group labelled*

"Inactive ASO; IT" with high pNFH levels.

3.5. Quantitative PCR analysis showed that intrathecal delivery of SOD1-targeting antisense oligonucleotide achieved approximately 60% knockdown of human SOD1 mRNA in spinal cord tissue [1], whereas intracerebroventricular delivery resulted in approximately 35% knockdown [2], both significantly different from the inactive control [3]

3.5.1. LAB MEDIA: Figure 3. *Video editor: Highlight the green triangle bar for "SOD1-ASO 100 µg; IT" showing the lowest SOD1 mRNA levels.*

3.5.2. LAB MEDIA: Figure 3. *Video editor: Highlight the blue circle bar for "SOD1-ASO 100 µg; ICV" showing moderate SOD1 mRNA reduction.*

3.5.3. LAB MEDIA: Figure 3. *Video editor: Highlight the red square bar for "Inactive ASO; IT" showing the highest SOD1 mRNA expression*

Pronunciation Guide:

Intrathecal

Pronunciation link: <https://www.merriam-webster.com/dictionary/intrathecal>
IPA: /ˌɪntrəˈθiːkəl/
Phonetic Spelling: in·truh·thee·kuhl

SOD1

Pronunciation link: No confirmed link found
IPA: /ˌɛs ɒʊ diː ˈwʌn/
Phonetic Spelling: ess·oh·dee·wun

Antisense

Pronunciation link: <https://www.merriam-webster.com/dictionary/antisense>
IPA: /ˌæntaɪˈsens/
Phonetic Spelling: an·tye·sens

Oligonucleotides

Pronunciation link: <https://www.merriam-webster.com/dictionary/oligonucleotide>
IPA: /ˌɑːlɪɡoʊˈnuːkliəˌtaɪdz/
Phonetic Spelling: ol·ih·goh·noo·klee·uh·tydz

Cerebrospinal

Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebrospinal>
IPA: /səˌriːbroʊˈspaɪnəl/
Phonetic Spelling: suh·ree·broh·spy·nuhl

Intracerebroventricular

Pronunciation link: No confirmed link found
IPA: /ˌɪntrə səˌriːbroʊvenˈtrɪkjələr/
Phonetic Spelling: in·truh·suh·ree·broh·ven·trik·yuh·ler

Anatomical

Pronunciation link: <https://www.merriam-webster.com/dictionary/anatomical>
IPA: /ˌænəˈtɑːmɪkəl/
Phonetic Spelling: an·uh·tom·ih·kuhl

Cistern

Pronunciation link: <https://www.merriam-webster.com/dictionary/cistern>
IPA: /ˈsɪstərn/
Phonetic Spelling: sis·tern

Ophthalmic

Pronunciation link:
[webster.com/dictionary/ophthalmic](https://www.merriam-webster.com/dictionary/ophthalmic)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/ophthalmic)

IPA: /ɑːfˈθælmɪk/

Phonetic Spelling: off-thal-mik

Desiccation

Pronunciation link:
[webster.com/dictionary/desiccation](https://www.merriam-webster.com/dictionary/desiccation)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/desiccation)

IPA: /ˌdɛsɪˈkeɪʃən/

Phonetic Spelling: des-ih-kay-shuhn

Buprenorphine

Pronunciation link:
[webster.com/dictionary/buprenorphine](https://www.merriam-webster.com/dictionary/buprenorphine)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/buprenorphine)

IPA: /ˌbjuːprəˈnɔːrfiːn/

Phonetic Spelling: byoo-pruh-nor-feen

Povidone-Iodine

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

IPA: /ˈpɑːvɪˌdoʊn/

Phonetic Spelling: pov-ih-dohn

Intervertebral

Pronunciation link:
[webster.com/dictionary/intervertebral](https://www.merriam-webster.com/dictionary/intervertebral)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/intervertebral)

IPA: /ˌɪntərˈvɜːrtəbrəl/

Phonetic Spelling: in-ter-vur-tuh-bruhl

Dura Mater

Pronunciation link:
[webster.com/dictionary/dura%20mater](https://www.merriam-webster.com/dictionary/dura%20mater)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/dura%20mater)

IPA: /ˌdʊrə ˈmeɪtər/

Phonetic Spelling: dur-uh may-ter

Intrathecal

Pronunciation link:
[webster.com/dictionary/intrathecal](https://www.merriam-webster.com/dictionary/intrathecal)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/intrathecal)

IPA: /ˌɪntrəˈθiːkli/

Phonetic Spelling: in-truh-thee-kee

Rostrocaudal

Pronunciation link:
[webster.com/dictionary/rostromedial](https://www.merriam-webster.com/dictionary/rostromedial)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/rostromedial)

IPA: /ˌrɑːstroʊˈkɔːdəl/

Phonetic Spelling: ros-troh-kaw-duhl

Intracerebroventricularly

Pronunciation link: No confirmed link found

IPA: /ˌɪntrə səˌriːbrʊvənˈtrɪkjələrli/

Phonetic Spelling: in·truh·suh·ree·broh·ven·trik·yuh·ler·lee

Phosphorylated

Pronunciation link:
[webster.com/dictionary/phosphorylated](https://www.merriam-webster.com/dictionary/phosphorylated)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/phosphorylated)

IPA: /ˈfɒːsfəˌreɪtɪd/

Phonetic Spelling: fos·fuh·ray·tuhd

Neurofilament

Pronunciation link:
[webster.com/dictionary/neurofilament](https://www.merriam-webster.com/dictionary/neurofilament)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/neurofilament)

IPA: /ˌnʊrəʊˈfɪləmənt/

Phonetic Spelling: nur·oh·fil·uh·ment

Quantitative

Pronunciation link:
[webster.com/dictionary/quantitative](https://www.merriam-webster.com/dictionary/quantitative)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/quantitative)

IPA: /ˈkwɑːntəˌteɪtɪv/

Phonetic Spelling: kwon·tuh·tay·tiv

Knockdown

Pronunciation link:
[webster.com/dictionary/knockdown](https://www.merriam-webster.com/dictionary/knockdown)

[https://www.merriam-](https://www.merriam-webster.com/dictionary/knockdown)

IPA: /ˈnɔːkˌdaʊn/

Phonetic Spelling: nok·down