Protocol Detail Report Answered Questions Only

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Report Comments

Protocol Information

Version # 3

Reference Number	200478		
Protocol Number	16-200478-HSC		
Protocol Type:	Renewal		
Principal Investigator:	[redacted]	**Approval Date:**	3/8/2018
Submittal Date:	3/7/2018	**Effective Date:**	3/8/2018
Author:	[redacted]	**Renewal Date:**	3/8/2019
Status:	Approved	**Next Review Date:**	3/8/2019
Inactive Date:		**Expiration Date:**	3/8/2019

Annual Renewal Section

Continuation Status 1.1

Do you wish to continue this protocol?

- [x] Yes
- [ ] No

1.1.1

Yes

Annual Renewal Progress Report 1.1.1.1

Give a brief summary of progress made during the past year.
Include species and number of animals used.

The data obtained from the experiments performed in 2016 resulted in publication: [redacted] No animals have been used in 2017.

Expected Progress in Coming Year 1.1.1.2

Briefly define research goals projected for completion in the upcoming year.

This year, we will continue our studies in the same direction and investigate the role of microRNAs in post-stroke recovery. dMCAO Mouse model of stroke will be utilized.

Complications Associated with Animal Procedures 1.1.1.3

Have technical complications occurred with animal procedures in the past year?

- [ ] Yes
- [x] No

1.1.1.3.2

No

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Funding Status 1.1.1.4

Have there been any changes in funding for this project?

- [ ] Yes
- [x] No

1.1.1.4.2

No

Current Field Permits 1.1.1.5

Are all your field permits up to date (if applicable)?

- [ ] Yes
- [ ] No
- [x] N/A

1.1.1.5.3

N/A

Updated or New Animal Handling Training and Medical Clearance 1.1.1.6

All protocol associates must be medically cleared annually and appropriately trained to handle animals.

Please verify all protocol associates listed on this protocol are up to date on their medical clearance and training.

Renewal Type 1.1.1.7

Select one. If you wish to make changes at this time (ex: add/remove staff) select "Annual Renewal/Minor Amendment"
If you want to submit a Major Amendment, please submit it after this annual renewal is approved.

- [ ] Annual Renewal ONLY
- [x] Annual Renewal/Minor Amendment

1.1.1.7.2

Annual Renewal/Minor Amendment

Minor Amendment Change(s) 1.1.1.7.2.1

Select ALL that apply.

- [x] Add/Remove Staff
- [ ] Increase of animals less than 10%
- [ ] Minor procedural changes

1.1.1.7.2.1.1

Add/Remove Staff

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Add/Remove Staff 1.1.1.7.2. 1.1.1

Fill in the following. Only enter the email address and contact number if ADDING staff.
Click the green plus icon to the right to add rows to the table.

Add or remove the staff in the Administrative Section under "Protocol Associates".

Name	Email Address	Contact Number	Add/Remove
[redacted]			Remove

Administrative Data

Reference Number 2.1

This number is automatically populated by the system.
200478

Protocol Number 2.2

Protocol number is assigned by the Office of Animal Care Compliance (OACC) upon approval.
16-200478-HSC

Title 2.3

Maximum limit is 255 characters, be concise.
Note: The title should include reference to procedures and the animal species to be used (e.g. "A Rat Model of Ischemic Stroke.").
Regeneration after stroke and brain trauma

Principal Investigator 2.4

PI - individual solely responsible for the protocol, its assurances, and can order animals.
Click on the silhouette with the green plus icon below to select the PI. You may type the PI name in the search window.

Select the blue information icon to review your contact information. If it needs updating please email HSC-OACC@salud.unm.edu

[redacted]

Department 2.5

Select the appropriate department from the drop down list by clicking the green plus icon to the right.
Click the "+" or "-" to expand or collapse the entire list of departments or type the department name in the search window then select "OK".
HSC

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Author 2.6

Someone who fills out or works on the request, and is authorized to order animals.
Click on the silhouette with the green plus icon below to select the Author. You may type the Author name in the search window.

[redacted]

Co-Author 2.7

This person should be someone other than the PI who can access and edit the protocol in the PI's absence.
Click on the silhouette with the green plus icon below to select the Co-Author. You may type the Co-Author name in the search window.

[redacted]

Created By 2.8

Creator - the person who initially creates the protocol (auto-populated per the login and cannot be changed) and who may populate documents.

[redacted]

Protocol Associates 2.9

Personnel who will perform animal work or will handle animal blood or tissue.
Select the appropriate department from the drop down list by clicking the green plus icon to the right.

You may type the Protocol Associate name in the search window and/or check the box to choose that associate then select "OK".

*In the responsibilities field, indicate if one of the protocol associates is also the Project Coordinator.
There are three different types of personnel who may be working with animals:
Co-Investigator - someone who is able to alter this protocol on your behalf.
Key Associate - someone who will receive email notifications on status of protocol.
Authorized to Order Animals - someone who may order animals for this protocol on your behalf.

[redacted]

Responsibilities	All animal/surgery procedures
Comments	

☐ **Co-Investigator** ☐ **Key Associate** ☑ **Authorized to Order Animals**

Animal Handling Training 2.10

[redacted]

Attach the Personnel Qualifications Form here for the PI and all protocol associates by clicking on the paperclip icon to the right:
Personnel Qualifications Form - training for specific hands-on procedures performed on LIVE VERTEBRATE ANIMALS at UNM (this form is NOT needed for collaborative, display, holding, or tissue protocols).
Protocol associates who are not properly trained may not handle animals and may not be listed on approved protocols.
Click on the links below to access either the Personnel Qualifications Form (hands-on training) or the OACC training website (disable popups on your browser to view attachments).

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3 Year Re-Write 2.11

Does this replace an expiring, expired, or a one-year transfer protocol?

- [x] Yes
- [ ] No

2.11.1

Yes

Source Protocol 2.11.1.1

Select the UNM protocol this is replacing from the list by clicking the green plus icon to the right.
You may type any part of the protocol information (protocol number, title, PI, etc.) in the search window.

Reference #	Protocol #	Status	Title	Type
200207	13-100956-TR-HSC	Approved	Regeneration after stroke and brain trauma	Original

Progress Report 2.11.1.2

Please provide a brief progress report relating to research during the previous approval period.
Identify the studies described in the previously approved protocol that have already been completed.
Indicate the numbers of animals by species that have already been used.
Indicate if there were any adverse events that occurred and explain how they were addressed.
Reference publications generated out of this research.
Describe how the work proposed in this new protocol will extend the previous studies.

The number of animals used is the following: Mouse category D: 16 used.
Our objective was to study the effect of microRNAs on the regeneration after stroke. The data obtained during previous and current approval periods were used for NIH R01 annual reports (funded). Our study resulted in two publications:

1) [redacted]

2) [redacted]

The work proposed in this new protocol will be a continuation of our earlier studies. We will use the described animal models for more detailed investigation of regeneration processes after stroke and brain trauma

Associated Protocols 2.12

Are there any associated protocols (ex: breeding or collaborative)?

- [ ] UNM protocols for which you are associated (as PI, Co-PI, Author, Creator, etc.)
- [ ] UNM protocols for which you are not associated (but that you may transfer animals to, etc.)
- [ ] External related protocols
- [x] None

2.12.4

None

Office Use Only

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Medical Clearance to handle animals, their blood, tissues, or cell lines 3.1

Have the PI and/or all protocol associates been medically cleared to handle animals, their blood, tissues, or cell lines?
All protocol associates must be medically cleared ANNUALLY. Associates who are not medically cleared may not handle animals and may not be listed on approved protocols.
Office Use Only - checked by [redacted]. Cannot be filled out by PI.

- [x] Yes
- [ ] No

3.1.1

Yes

Online Animal Handling Training 3.2

Have the PI and/or all protocol associates completed the required online animal handling courses in the AALAS Learning Library (ALL)?
Office Use Only - checked by [redacted]. Cannot be filled out by PI.

- [x] Yes
- [ ] No

3.2.1

Yes

Grant Congruency Review 3.3

Is the relevant information in this protocol congruent with the attached vertebrate animal section(s) of the PHS funded grant(s)?
Office Use Only - grant congruency review performed by [redacted]. Cannot be filled out by PI.

- [x] Yes (required for PHS funded)
- [ ] No (not required for non-PHS funded)

3.3.1

Yes (required for PHS funded)

Scientific Merit

Funding 4.1

Is this project funded?

- [x] Yes
- [ ] No

4.1.1

Yes

Funding Source 4.1.1.1

Select all funding sources by clicking on the green plus icon to the right.
If your funding source is not listed here, please email HSC-OACC@salud.unm.edu and we will add it for you.

You may click the green plus sign to choose that funding source then select "OK".

NIH/NINDS

4.1.1.1.1

NIH/NINDS

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Grant Information and FP# **4.1.1.2**

Fill in the following.

Click the green plus icon to the right to add rows to the table.

Title of grant if different from protocol	Grant number/identifier	Grant start date	Grant end date	UNM Identifier (ex: Cayuse#/HSC Pre-Award FP#)
In vivo Inhibition of Specific microRNAs to Support Post-Stroke Revascularization	[redacted]	09/01/2013	08/31/2018	[redacted]

PHS Funding **4.2**

Is this project Public Health Service (PHS) funded?
PHS agencies = National Institutes of Health (NIH), National Science Foundation (NSF), plus the following: ACF, AoA, AHRQ, ATSDR, CDC, CMS, FOH, FDA, HRSA, IHS, SAMHSA

- [x] Yes
- [ ] No

4.2.1

Yes

Grant Congruency **4.2.1.1**

Attachments: ANIMALS.pdf

Congruency review is required for all PHS agencies = NIH plus the following: ACF, AoA, AHRQ, ATSDR, CDC, CMS, FOH, FDA, HRSA, IHS, SAMHSA
*Attach the relevant vertebrate animal section(s) of the grant(s) to document congruency between the animal protocol and the vertebrate animal section(s) of the grant by clicking the paper clip icon to the right.

Conflicts of Interest **4.3**

Has a Conflict of Interest (COI) form been submitted to the Pre-Award Office for grants associated with this protocol?

- [x] Yes
- [ ] No

4.3.1

Yes

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Scientific Abstract/Project Overview 4.4

Attachments: Biosketch Roitbak 2016 copy.docx

Include aims/hypotheses/objectives.
The PI may choose to add a link to their NIH Biosketch, Researchgate, research page, or other summary of relevant publications by clicking on the paperclip icon to the right or embedding a link in the text below.

MicroRNAs are recently descovered class of small non-protein coding RNA molecules implicated in wide range of gene regulatory mechanisms. Literature data demonstrate that numerous microRNAs are expressed in the nervous system and play important role in brain regeneration. In ourexperiments, we identified the subset of endothelial cell (EC) microRNAs significantly affecting capillary formation in vitro. We found that these microRNAs are regulating EC morphogenesis. To validate our in vitro experiments and most importantly, find a possible clinical application of our findings, we perform an in vivo delivery of the specific microRNA inhibitors into the mouse vasculature. We expect that this procedure will improve the brain vascularization following stroke.

Lay Summary 4.5

Address species or taxon of animal(s) using common names, goals, a list of animal procedures, and benefits of the study.
Use non-scientific words.
Eliminate or define abbreviations, technical terms, and jargon.
Limit 500 words.

Stroke-induced ischemic brain injury involves significant damage to cerebral vasculature which results in neuronal damage and cell death. Functional recovery after cerebral ischemia is highly dependent on the effective restoration of blood supply to the damaged brain area. Therefore extensive investigation aiming to promote recovery following stroke is concentrated on post-stroke angiogenesis. Our long-term goal is to develop a method for supporting the revascularization process following stroke, in order to improve post-injury recovery of the brain. We propose that brain recovery process can be supported via the regulation of small molecules called microRNAs.

Scientific Merit Review 4.6

Was this project subject to independent peer review?

- [x] Yes
- [ ] No

4.6.1

Yes

Scientific Reviewing Entity 4.6.1.1

List the scientific reviewing entity.

NIH

Alternatives Search

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The 3 Rs Principle of Animal Welfare in Research 5.1

Attachments: altwksht.pdf

See attached Animal Welfare Information Center (AWIC) worksheet below if you want additional guidance in completing your alternative search.
The Animal Welfare Act and PHS (NIH) policy for using animals in research and teaching require that the PI has adequately addressed attention to improving animal welfare in laboratory settings. The 3R's principle summarizes these concerns.

REPLACEMENT of animals with non-animal methods and choosing the phylogenetically lowest species whenever possible is essential. Scientists are requested to establish that animals are necessary to reach the scientific objective. It is imperative that scientists determine that alternatives to using the proposed species to achieve the scientific objective are not available. [Mice and rats are generally considered the lowest mammalian species, if it is necessary to conduct the study in mammals. However, invertebrates, amphibians, fish and insects represent lower species that may be considered in some studies].

REDUCTION of the number of animals to that which is the least number required to achieve the scientific objective is the second premise. If animals are necessary for the experiment, then adequate justification of the proposed number is required.

REFINEMENT of all procedures employed in the protocol to minimize pain, stress, and distress is a major consideration. This principle applies to procedures classified as category D or E, i.e., wherein more than momentary pain or distress is possible. Once the scientist has determined that the use of animals is essential, it is imperative that she/he investigate alternative and improved animal procedures that will alleviate pain or suffering. Additionally, the use of analgesics, sedatives, and other methods for addressing the animal's welfare must be explored.

Rationale for Vertebrate Animals and Species 5.2

Why is it necessary to use a vertebrate animal model? Why are the proposed species most appropriate?
Each species should be the lowest on the phylogenetic scale required to accomplish the research goal(s).

We plan to use rodents in our experiments, to be able to 1) use all accumulated knowledge in these field for the interpretation of our results, as well as 2) for a possible relevance of our findings to clinical application in humans. In our research we adhere to RRR principle of the laboratory animal use. We propose to minimize the animal pain and stress in all our procedures, and minimize the amount of the animals used: we will use the same animals for all imaging procedures, and keep the same animals for the evaluation at different time points after ischemia. We also established a cell culture method to model the blood brain barrier, so no additional animals will be needed to study the cell signaling pathways and molecular mechanisms. In-vivo experimental model of stroke will be often replaced by in-vitro oxygen-glucose deprivation. In summary, most of our work is performed in vitro, however, some experiments must be performed in vivo to validate the results. Non-animal computer-generated models cannot be used because they cannot reflect dynamics of the brain environment and vasculature in vivo during the brain regeneration.

Key Words for Unnecessary Duplication Search 5.3

PIs are required to assure the Committee that they have considered whether or not proposed animal work unnecessarily duplicates existing knowledge.
List the key words and combining terms that you used in your search in the text box.

miR-155 and stroke
In vivo inhibition of miR-155
In vivo microRNA delivery in mice.

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General Scientific Literature Databases Searched for Unnecessary Duplication 5.4

Attachments: Link to PubMed

Search PubMed or similar (live link below in blue) then list the database(s) searched.

NIH OACU
PubMed

Search Date for Unnecessary Duplication 5.5

Select the date of your search.

2/12/2016

Search Period for Unnecessary Duplication 5.6

Period (years) covered by each search (it is recommended that for new projects your search covers the last 20 years).

2005-2016

USDA Pain or Distress Categories D or E 5.7

Do any animals on this protocol fall under USDA pain or distress categories D or E?
See help ? for more information.

Category D - Alleviated Pain
Category E - Unalleviated Pain

- [x] Yes
- [ ] No

5.7.1

Yes

Alternatives to Potentially Painful/Distressful Procedures Search 5.7.1.1

The goals of alternatives to painful or distressful procedures are:
· to find methods that use non-animal systems
· to use the least sentient animal species
· to use methods that refine animal use by lessening or eliminating pain or distress, thereby enhancing animal well-being
· to use the minimum number of animals necessary to attain research objectives

If potential alternatives DO NOT allow the attainment of the goals of the research then they are not valid alternatives

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Key Words for Alternatives to Potentially Painful/Distressful Procedures 5.7.1.2

Attachments: Alternatives Search Website

Perform one or more database searches for alternatives to painful/distressful procedures for each of the databases listed below following the examples in the Alternatives Search Website link:

List the key words and combining terms that you used in your search in the text box.

Minimally invasive procedure AND mouse AND cerebral ischemia
Rodent stroke AND modeling brain regeneration
Stroke AND Hypoxia AND in vitro model
mouse AND MCAO
Mouse AND MCAO AND craniotomy
Distal MCAO AND survival AND mouse

Scientific Databases Searched for Alternatives to Potentially Painful/Distressful Procedures 5.7.1.3

Attachments: PubMed TOXNET

Search at least one of the following databases (live links to databases are in blue) then list the database(s) searched.

PubMed, TOXNET

Alternatives to Potentially Painful/Distressful Procedures Databases Searched 5.7.1.4

Attachments: Link to AGRICOLA Link to AWIC and ALTWEB

Search at least one of the following databases (live links to databases are in blue) then list the database(s) searched.

AGRICOLA, AWIC, ALTWEB, and CAAT

Search Date for Alternatives to Potentially Painful/Distressful Procedures 5.7.1.5

Date of Alternative Search
2/15/2016

Search Period for Alternatives to Potentially Painful/Distressful Procedures 5.7.1.6

Period (years) covered by each search (it is recommended that for new projects your search covers the last 20 years).

2005-2015

Unalleviated Pain (Category E) 5.7.1.7

Are any animals subjected to unalleviated pain or distress?

- ☐ Yes
- ☑ No

5.7.1.7.2

No

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Alternative Search Results 5.8

Please select ALL that apply and provide narrative as indicated.

- [ ] I found evidence that completion of this study might lead to UNECCESSARY duplication.
- [ ] I identified a non-animal model or a less sentient species that could be used for my research.
- [ ] I found ways to reduce my animal numbers.
- [ ] I found other methods that minimize or replace the potentially painful or distressful procedures in this animal protocol.
- [x] I found no viable alternatives (refinement, replacement, reduction) as listed above.

5.8.5

I found no viable alternatives (refinement, replacement, reduction) as listed above.

Alternative Search Results Narrative 5.9

Provide a narrative of your search results. Give a detailed explanation of any checked statements.

Our rearch goal is to show that proposed treatment results in the improvement of the animal functional recovery, which has to be demonstrated with behavioral tests. Animal model of experimental ischemia is needed in these studies because there is no alternate substitute (e.g., in vitro cell culture system; computer modeling) for the complexity of the animal behavior. Off the known mouse ischemia models, distal middle cerebral artery occlusion is a minimally invasive procedure, which is characteried by high reproducibility and low mortality of the animals. The animals do not show any signs of distress within several hours of the procedure. The induced brain damage is very mild and is not associated with any visible disabilities in the animal. Because the craniotomy is minimally invasive, the animal recovery is very rapid.

Species

Animal Source 6.1

Check all that apply.

- [ ] Captured/Collected/Observed in the Field
- [x] External Source (Commercial Vendor or Non-commercial)
- [ ] In-house Breeding or Transfer from an Internal Protocol

6.1.2

External Source (Commercial Vendor or Non-commercial)

Commercial or Non-commercial Animal Source 6.1.2.1

Select all that apply.

All animal orders must be approved in advance by the Animal Resource Facility (ARF) office.

- [x] Approved Commercial Vendor [redacted]
- [ ] Non-commercial Vendor (e.g. Universities, Research Institutions, etc.)

6.1.2.1.1

Approved Commercial Vendor [redacted]

Acclimation 6.1.2.2

Will animals be given an acclimation period of at least 3 days upon receipt prior to invasive procedures? Non-invasive procedures such as body weight measurements can be conducted during this period.

- [x] Yes
- [ ] No

6.1.2.2.1

Yes

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Acclimation Description 6.1.2.2.1.1

How long will animals be acclimated following receipt or significant change in environment? Animals should be acclimated for 3 days or longer depending on the species and type of research. Describe any baseline non-invasive procedures performed during the acclimation period such as weighing, etc.

After the animals are received, the are allowed to acclime for one week, before they are used in any procedure, includng surgery, injections, or behavioral tests

Methods for Individual Animal Identification 6.2

Cage cards are always required for animals housed in UNM Animal Research Facilities (ARFs). Check all other methods that apply.

- [x] Bands or Tags
- [ ] Ear Punch
- [ ] Fur Dye or Marker
- [ ] Microchip/Transponder
- [ ] Tattoo
- [ ] Other

6.2.1

Bands or Tags

Live Animal Non-commercial Transport 6.3

Will live animals be transported non-commercially (including on campus)?
Transportation of live animals must conform to all institutional guidelines/policies and federal regulations.

- [ ] Yes
- [x] No

6.3.2

No

Special Husbandry 6.4

Will any live vertebrate animals be subjected to special husbandry such as:
Food or drinking water manipulations
Fluid or food restrictions
Lack of enrichment
Modified light cycle
Single housing of social animals
Special health monitoring
Special caging (metabolic cage, suspended wire floor, etc.)
Temperature extremes
Unusual means of identification

- [ ] Yes
- [x] No

6.4.2

No

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Prolonged Restraint 6.5

Will live vertebrate animals be subjected to prolonged restraint?

- [ ] Yes
- [x] No

6.5.2

No

Habituation 6.6

Will animals be habituated to novel experimental procedures that have an increased potential for pain or distress (e.g., behavioral testing, handling/restraint, tube restraint, etc.)?
If not applicable, select "No" and state accordingly.

- [x] Yes
- [ ] No

6.6.1

Yes

Habituation Description 6.6.1.1

Give a narrative of your habituation procedures.

The animals are habituated to the behavioral procedures and behavior equipment one week before surgery. They are also habituated to the testing room for 30 min, everytime before testing. behavioral tests that we use are not associated with any pain or distress to the animal.

Paralytic Use 6.7

Will you administer paralytics (neuromuscular-blocking drugs) to live vertebrate animals?

- [ ] Yes
- [x] No

6.7.2

No

Tissue Transplantation 6.8

Will you transplant tissues into live vertebrate animals?

- [ ] Yes
- [x] No

6.8.2

No

Antibody Production by Ascites 6.9

Will you produce antibodies by ascites?

- [ ] Yes
- [x] No

6.9.2

No

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Pain Management 6.10

Do any of the procedures on this protocol potentially result in pain/distress?

- [x] Yes
- [ ] No

6.10.1

Yes

List Potentially Painful/Distressful Procedures 6.10.1.1

List the potentially painful/distressful procedures or outcomes.

The animal might feel the pain immediately after surgery

Alleviation of Pain/Distress? 6.10.1.2

Will alleviation of pain/distress be provided?

- [x] Yes
- [ ] No

6.10.1.2.1

Yes

Description of Alleviation of Pain/Distress 6.10.1.2.1.1

Describe how pain/distress will be alleviated (to include anesthesia/analgesia, early removal, etc.).

The animals will be anesthetized with isoflurane and O2:N2O mixture duing the surgery and life imaging procedures.

Local anesthetic/analgesics: Lidocaine hydrochloride5 mg/kg total dose, intra-incisional , will be used locally before making surgical incision
Pre-emtive analgesia: Buprenorphine 0.1mg/kg, immediately before the surgery and then at 4-8 hours after surgery.

Significant Adverse Clinical Conditions 6.11

Do you expect onset of any morbidity (unhealthiness, illness, or adverse clinical conditions) in these animal models as a result of either the research procedures or the animals' phenotype?

- [x] Yes
- [ ] No

6.11.1

Yes

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Expected Adverse Outcomes 6.11.1.1

Select all that apply.
If severe outcomes are expected you may be required to utilize a clinical scoring sheet (see post-procedural monitoring).

- [ ] Animals expected to die?
- [x] Poten ial for animals to die as a result of manipulations?
- [ ] Animals expected to become dehydrated?
- [x] Poten ial for dehydration as a result of manipulations?
- [ ] Animals expected to become moribund?
- [ ] Poten ial for morbidity as a result of manipulations?
- [ ] Animals expected to lose weight?
- [x] Poten ial for weight loss as a result of manipulations?
- [ ] Other

6.11.1.1.2

Potential for animals to die as a result of manipulations?

6.11.1.1.4

Potential for dehydration as a result of manipulations?

6.11.1.1.8

Potential for weight loss as a result of manipulations?

Actions for Severe Clinical Signs 6.12

Severe clinical signs (e.g. moribund or unresponsive when stimulated, severe dehydration, weight loss > 15-20%, severe dyspnea, severe neurological, evidence of intractable pain, deep ulcerated skin associated with skin or subcutaneous tumors) indicate aggressive action is necessary, including euthanasia.
*If any of severe signs are present, will immediate treatment or euthanasia be elected?

- [x] Yes
- [ ] No

6.12.1

Yes

Actions for Persistent Clinical Signs 6.13

Persistent or progressive signs of illness beyond 24-48 hours(e.g. decreased appetite, decreased activity, irritable or aversion to handling that may be associated with pain, shivering, piloerection, hunched, progressive breathing or neurological anomalies, tumors that exceed 10% of body weight or >2 cm mice; > 4 cm rats, loss of body condition, or other clinical conditions that are progressive and/or unresponsive to treatment),may indicate aggressive action is necessary, including altered treatment or euthanasia.

*If these signs persist beyond 48 hours, or worsen within a 24-48 hour period, will euthanasia be elected?

- [x] Yes
- [ ] No

6.13.1

Yes

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Species Selection **6.14**

Add all appropriate species for this study or class of animals for a field study by clicking on the green plus icon on the right.
You may click the green plus sign to choose that species then select "OK".

After you've made your selection, click on the blue species "segment" to access or complete information about animal numbers and procedures.

6.14.1

Mouse #1

6.14.1

Mouse #1

Species Description **6.14.1.1**

Use this identifier only if the protocol consists of separate, relatively unrelated studies using the same animal species, for multiple projects, or for listing individual species in field studies.
Otherwise leave this blank.
*Strains are listed below.
C57BL/6 Mice

Strain/Stock/Breed **6.14.1.2**

Select the Strain/Stock/Breed from the drop down menu by clicking on the green plus icon on the right.

You may click the green plus sign to choose the strain then select "OK".

If your strain is not listed here, please email HSC-OACC@salud.unm.edu and we will add it for you.

C57BL/6

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Project Design and Experimental Flow 6.14.1.3

Attachments: Animal Numbers Table Template.xlsx

Start with the goal(s) of the proposed work as they relate to the Overview and Objectives.
For protocols with more than a single aim using this species, designate each aim with a sub-project or aim #, and conform to this designation for the subsequent text and for the Animal Numbers Table (attached).
For each sub-project or aim describe the sequence of events/procedures and interval between procedures to help the reviewers understand what will happen to the animals from the time they enter the experiment until the final disposition of the animal.
For complicated experimental designs flow charts or diagrams are strongly recommended. The attached Animal Numbers Table Template should generally be used as well.
Once the table is completed, attach it using the paperclip icon to the right.

Detailed descriptions of animal procedures are requested later.

1) dMCAO procedure: The MCA is exposed via transtemporal approach. A small burr hole (located 1 mm rostral to the fusion of zygoma and squamosal bone, and 3 mm ventral to the parietal bone) was made on the left side of the skull surface, and the MCA was coagulated with low-heat electrocautery ([redacted]). In sham-operated animals, the MCA is exposed but not coagulated.

2) Injection of (ethynyl deoxyuridine) EdU into the intraperitoneal cavity: Generation and maturation of the neuronal progenitors will be detected using Edu injections (intraperitoneal .6mg/100 micorliters/injectionis 100 microliters) performed twice a day, for three consecutive days, followed by a variable survival time allowing for tracking the fate of divided cells and their progeny. At the end of the experiment (at 7 days post MCAO), the animal is sacrificed and the tissues fixed with a standard paraformaldehyde-based fixative. As suggested by the IACUC, we will start to use EdU instead of BrdU, which is not genotoxic because it does not intercalate into the DNA.

3) In vivo delivery of microRNA inhibitors
Intra-venous delivery of specific miRNA inhibitors will be performed at 3 days following dMCAO, and the brain microvasculature will be analyzed at different time points (7, 14, and 21 days) following injections. Injection dose is 10 mg/kg (according to manufacturers recommendations) for 3 consecutive days. Injections will be made via lateral tail vein, 100 microliters of total volume will be injected. The goal of this aim is to determine the molecular mechanisms of NSPC-induced vasculotrophic signals in angiogenesis after stroke. In the amendment dated by March 2015, we request more animals in order to perform additional experiments on the effect of in vivo microRNA inhibition on post-stroke inflammation. In vivo delivery of microRNA inhibitors will be performed similarly, and at the same time-points as was proposed earlier in the present protocol.

4) In vivo imaging: At 24 hors, 7, 14 and 21 days following dMCAO, the animals will be subjected to in vivo two-photon microscopy imaging (40 min – 1h under anesthesia) or MRI imaging (40 mon-1 hour of anesthesia)

5) Behavioral testing (adhesive removal test and CatWalk test) will be performed before and after dMCAO regularly. The days when the in vivo imaging is performed, will be skipped.

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Group Size Justification Statement 6.14.1.4

Experimental designs should clearly define various groups necessary to support each aim. Each unique group in the experiment should be defined; and methods used to determine the group size discussed, including prior experience that inform statistical analyses.

When possible the statistical method should be used (e.g., power analysis, including the variable employed).

If scientific, statistical renderings are inappropriate, or adequate data are unavailable to evaluate in this manner, clarify other means used to make group size determination. It is important that actual numbers in experimental groups be justified using the best information available to you at this time.
The checklist below provides some standard criteria used to determine minimum numbers of animals required per group as necessary to achieve objectives. Please select all that apply and answer applicable questions.
Note: The "reduction principle" of animal experimentation can be violated by having too few animals in a group size if no useful information is obtained.

Justification Checklist 6.14.1.5

Check all of the following that apply and answer respective questions.

- [x] Group sizes determined statistically
- [ ] Group sizes based upon quantity of harvested cells or tissue required for in vitro studies
- [ ] Number prescribed for contract study
- [ ] Pilot or preliminary project; group variances unknown at present.
- [ ] Other

6.14.1.5.1

Group sizes determined statistically

Group Size Statistical Explanation 6.14.1.5.1.1

Describe the statistical method used to support the group sizes for each research aim that applies under this species section.
*Attach a Power Analysis if possible.

A power analysis was used to identify the minimal number of animals needed to avoid a Type II Error. The assumptions for our power analysis are: a fixed a level of 0.05 with a planned two-tailed pair-wise comparison, standard deviation of 20 percent and a 50 percent difference between the groups is substantively important. Under these assumptions, a sample size of 20 per treatment condition will provide us above 95% power for significance determinations. (G. Keppel, Design and Analysis: A Researchers Handbook, 3rd ed, Prentice Hall, 1991.).

Authorized Amounts 6.14.1.6

Click on the green plus icon on the right to add the requested animal numbers by pain or distress category.
You may click the green plus sign to choose the proper USDA Pain Category then select "OK".

Pain Category	Authorized	Requested	On Order	Received	Adjustment	Available
USDA Category D - Alleviated	100	0	0	16	0	84
Totals	100	0	0	16	0	84

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Procedures 6.14.1.7

Select all procedures that apply by clicking on the green plus icon on the right.
You may click the green plus sign to choose the procedure then select "OK".

This section is required for reporting purposes.

6.14.1.7.1

Surgery - Survival

Associate Selection 6.14.1.7.1.1

Indicate who will perform the procedure(s) by clicking on the green plus icon to the right.

[redacted]

Location Selection 6.14.1.7.1.2

Indicate where the procedures will be performed (Building and Room) by clicking on the green plus icon to the right.

[redacted]

6.14.1.7.2

Imaging

Associate Selection 6.14.1.7.2.1

Indicate who will perform the procedure(s) by clicking on the green plus icon to the right.

[redacted]

Location Selection 6.14.1.7.2.2

Indicate where the procedures will be performed (Building and Room) by clicking on the green plus icon to the right.

[redacted]

6.14.1.7.3

Specimen Collection (other than blood)

Associate Selection 6.14.1.7.3.1

Indicate who will perform the procedure(s) by clicking on the green plus icon to the right.

[redacted]

Location Selection 6.14.1.7.3.2

Indicate where the procedures will be performed (Building and Room) by clicking on the green plus icon to the right.

[redacted]

6.14.1.7.4

Behavioral

Associate Selection 6.14.1.7.4.1

Indicate who will perform the procedure(s) by clicking on the green plus icon to the right.

[redacted]

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Location Selection 6.14.1.7.4.2

Indicate where the procedures will be performed (Building and Room) by clicking on the green plus icon to the right.

[redacted])

Procedure Types 6.14.1.8

Check all procedure types that you selected above.
This section is required for describing methods.

- [x] Anesthesia/Analgesia
- [x] Agent Administration/Dosing
- [ ] Blood Collection
- [x] Specimen Collection (other than blood)
- [x] Surgery
- [x] Imaging
- [x] Behavioral
- [ ] Other

6.14.1.8.1

Anesthesia/Analgesia

Anesthetic or Sedative Agent Table 6.14.1.8.1.1

Fill in the information below (required).

Click the green plus icon to the right to add rows to the table.

*In addition, you may attach an SOP if applicable by clicking on the paperclip icon to the right.

Agent	Initial Dose	Route	Volume	Frequency	Duration
Isoflurane	2-3%	Inhalation			during surgery 30 min
mixture of O2:N2O	2:1 ratio	Inhalation			during surgery 30 min

Anesthetic or Sedative Agent Description/Additional Procedures 6.14.1.8.1.2

Give a narrative of your procedures and explain any details not covered in the table above.

Isoflurane and O2:N2O mixure will be used during the MRI and live imaging for 30 minutes during each procedure

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Analgesic, Local Anesthetic, or Pain/Distress Management Table 6.14.1.8.1.3

Fill in the information below (required).

Pre-emptive analgesic/pain management is expected.

Click the green plus icon to the right to add rows to the table.

*In addition, you may attach an SOP if applicable by clicking on the paperclip icon to the right.

Agent	Initial Dose	Route	Volume	Timing (relative to painful procedure)	Frequency	Duration
buprenorphine	0.1 mg/kg body weight	subcutaneous		immediately before surgery + re-dose 4-8 hours after surgery. Second day after surgery: re-dose if signs of pain are evident		

Analgesic, Local Anesthetic, or Pain/Distress Management Description/Additional Procedures 6.14.1.8.1.4

Give a narrative of your procedures and explain any details not covered in the table above. If early removal criteria is used as pain management, state here and add details under post-procedural monitoring below.

If pre-emptive pain management cannot be provided for potentially painful procedures such as surgery it must be scientifically justified.

Local anesthetic/analgesics: Lidocaine hydrochloride5 mg/kg total dose, intra-incisional , will be used locally before making surgical incision.
Pre-emtive analgesia: Buprenorphine 0.1mg/kg, immediately before the surgery.
immediately before surgery + re-dose 4-8 hours after surgery. Second day after surgery: re-dose if signs of pain are evident

6.14.1.8.2

Agent Administration/Dosing

Agent Administration/Dosing Table 6.14.1.8.2.1

Fill in the information below (required).
Click the green plus icon to the right to add rows to the table.

*In addition, you may attach an SOP if applicable by clicking on the paperclip icon to the right.

Agent	Initial Dose	Route	Volume	Anesthesia Type	Frequency
specific or control microRNA inhibitor	10 mg/kg	lateral teil vein	100 microliters	3 consecutive days	not required
ethynyl deoxyuridine (Edu)	0.6 mg/100 microliters	intraperitoneal	100 microliters	2X per day, for 3 consecutive days	not required

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Agent Administration/Dosing Description/Additional Procedures 6.14.1.8.2.2

Give a narrative of your procedures and explain any details not covered in the table above.

For intravenous administration, the animals are restrained in a specially designed restrainer for maximum 5 minutes. No restrain is needed for intraperitoneal injections

Non-pharmaceutical Grade Agents 6.14.1.8.2.3

Will you administer any non-pharmaceutical grade agents to live animals (agents, diluent, etc.)?

- [x] Yes
- [ ] No

6.14.1.8.2.3.1

Yes

Non-pharmaceutical Grade Agents Description 6.14.1.8.2.3.1.1

Give a narrative of your use of non-pharmaceutical grade agents.
The explanation should include safety and efficacy standards related to the grade, purity, sterility, pH, pyrogenicity, compatibility, and pharmacokinetics of the chemical or substance to be administered.

microRNA inhibitors are routinely used in the animal experiments. The data safety and detailed description of these oligonucleotide probes, as well as IBC approval are provided in the biohasard section.

Non-pharmaceutical Grade Agents Justification 6.14.1.8.2.3.1.2

Explain why you are not using pharmaceutical grade agents.

No equivalent veterinary or human drug is available for experimental use of this agent. The reagent was formulated aseptically, no toxic vehicle is needed for the administration.

6.14.1.8.4

Specimen Collection (other than blood)

Specimen Collection (other than blood) Description 6.14.1.8.4.1

Give a narrative of your other specimen collection procedures (biopsy, tail snip, collection of excretions/secretions, etc). Include the anesthesia type if indicated or if tissue is collected after euthanasia.
*Including terminal specimen collection - specify terminal if applicable.

Tissue harvest after euthanasia: the brain, kidneys, heart and spleen will be collected after euthanasia.

6.14.1.8.5

Surgery

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Type of Surgery 6.14.1.8.5.1

Select all that apply.

- [ ] Non-survival Surgery
- [x] Survival Surgery

6.14.1.8.5.1.2

Survival Surgery

Aseptic Technique 6.14.1.8.5.1.2.1

Give a narrative of how you will ensure aseptic technique during survival surgery to include:
dedicated surgical area
skin preparation
surgical apparel
sterile instruments

All instruments will be autoclave-sterilized, the surgical table will be cleaned with disinfectant, and the surgeon will wear a face mask and surgical gloves and clothing. All surfaces on the surgical table will be covered with sterile cover. Surgical procedures will not be performed until the corneal and toe-pinch responses have been supressed. The fur around the surgical area will be clipped. The surgical area will be scrubbed with gauze, wiped with cotton-tipped applicators soaked in 70% ethanol , followed by 10% providone-iodine.

Type of Survival Surgery 6.14.1.8.5.1.2.2

Select all applicable types of survival surgery you will perform.

- [ ] Minor Survival Surgery
- [x] Major Survival Surgery
- [ ] Multiple Survival Surgery

6.14.1.8.5.1.2.2.2

Major Survival Surgery

Survival Surgery Description 6.14.1.8.5.1.2.3

Give a narrative of your survival surgery procedures. Include the anesthesia type if indicated.

[redacted]
A distal (direct) middle cerebral artery occlusion (dMCAO) is utilized as an experimental model of cortical ischemi. This model produces an infarct in the frontal and parietal cortex, which over time progresses into adjacent temporal, frontal, and cingulate cortex. Besides its high reproducibility and survival rate, the advantage of this model is that it produces smaller infarct comparable to human stroke. The MCA is exposed via transtemporal approach. A small burr hole (located 1 mm rostral to the fusion of zygoma and squamosal bone, and 3 mm ventral to the parietal bone) is made on the left side of the skull surface, and the MCA is coagulated with low-heat electrocautery (Bovie Medical). In sham-operated animals, the MCA is exposed but not coagulated. The skin is sutured with silk sutures and treated with the antibacterial oinment. Ophtalmic oinment is used during surgery and imaging, in order to prevent drying of cornea.
Anasthesia: Isoflurane: induction dosage 2-3%; maintenance dosage 1.5-2%), and a mixture of O2:N2O gases in the ratio 2:1, delivered during the procedure

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Potential Complications 6.14.1.8.5.1.2.4

Provide a narrative listing unexpected "worst case" complications that potentially could arise following ANY survival surgery or anesthesia.
This may include infection, wound dehiscence, unexpected reaction to the procedure or test substance
What action will be taken to address the issue. This may include acting at the advice of the attending veterinarian.

dMCAO will result in neurological impairment in mice, therefore they will be closely monitored and fed, as described in postoperative care. Worse case scenario includes intracerebral hemorrage and death after MCAO, however mice usually survives dMCAO procedure.

Post-operative Care 6.14.1.8.5.1.2.5

Provide a complete description of monitoring and care that will be provided to promote an uncomplicated recovery of the surgical patients, including:
Use of analgesics
Frequency and duration of monitoring
Supplementary thermal support & administration of parenteral fluids, other medications, etc.
Refer to the Surgery Guidelines or the veterinarian for additional information.
Post-operative time when sutures or wound clips are removed and monitoring is discontinued.

For thee experiments the mice will be kept in a standard animal room conditions, 2-4 animals per cage. No enriched environment will be provided, since the changes associated with the environment erichment will affect and complicate the rinterpretation of our results.
Subsequent to the surgical procedures, all animals receive treatment with analgesic (Rimadyl, 5 mg/kg of body weight). In addition, for 1 day after surgery, the specialized sucrolose gel cups (Clear H2O) containing oral analgesics (NSAIDs like carprofen) already dispersed in the cups.
The animals will be kept in the specially designated animal area at the [redacted].
Mice subjected to dMCAO will be monitored daily. Animals that appear hunched in posture, immobile or show any other signs of sickness or discomfort will be euthanized.
The mice will receive daily feeding by gavage 1X day of gerber baby formula: enfamil and gerber mixed baby cereal (mixed 1:1 with 2 parts water) and 1 ml of i.p. of sterile dextroze solution ([redacted]) for 7 days following surgery, with ad libitum access to hydrated rodent diet gel (veterinarian supplied).

6.14.1.8.6

Imaging

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Imaging Table 6.14.1.8.6.1

Fill in the information below (required).

Click the green plus icon to the right to add rows to the table.

*In addition, you may attach an SOP if applicable by clicking on the paperclip icon to the right.

Type	Duration	Anesthesia Type	#Images
MRI	30 minutes - 1 hour	induction dosage 2-3%; maintenance dosage 1.5-2%) and a mixture of O2:N2O gases in the ratio 2:1	10
Optical	30 min - 1 hour	induction dosage 2-3%; maintenance dosage 1.5-2%) and a mixture of O2:N2O gases in the ratio 2:1	10

Imaging Description/Additional Procedures 6.14.1.8.6.2

Give a narrative of your procedures and explain any details not covered in the table above.
If more than one trial per day, provide inter-trial interval. If more than one day, provide inter-session interval.

[redacted]. Anasthesia: Isoflurane: induction dosage 2-3%; maintenance dosage 1.5-2%), and a mixture of O2:N2O gases in the ratio 2:1, delivered during the procedure

6.14.1.8.7

Behavioral

Behavioral Procedures Type 6.14.1.8.7.1

Select all that apply.

- [ ] Attentional Set-Shifting Task
- [ ] Conditioned Place Preference
- [ ] Discrimination
- [ ] Fear Conditioning
- [ ] Forced Swim
- [ ] Light:Dark Box
- [ ] Maze
- [ ] Morris Water Task
- [ ] Nociception Testing
- [ ] Open Field Activity
- [ ] Motor Performance (e.g. Rotorod, Treadmill)
- [x] Other

6.14.1.8.7.1.12

Other

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Other Behavioral Procedures 6.14.1.8.7.1.12.1

Give a narrative of any additional behavioral procedures not previously described.
If more than one trial per day, provide inter-trial interval. If more than one day, provide inter-session interval.

Assessment of the animal functional recovery after dMCAO. Adhesive removal test: Bilateral asymmetry/Adhesive removal test is performed according to [redacted] l., 2009). Two adhesive tapes (~0.4 cm2) were applied with equal pressure on each animal paw. The order of placement of the adhesive (right or left) is alternated between each animal and each session. The mouse is placed in a transparent plastic box, and the times to contact and to remove each adhesive tape were collected, with a maximum of 120 s. Mice are trained daily before (5 days before surgery) and after dMCAO (reminder sessions at days 3-5 post-dMCAO). Post-surgery testing is monitored for 25 days after dMCAO (single trial per test day, the in-vivo imaging day was skipped). Two parameters are monitored for each paw: contact time (point that the mouse reacts to the presence of adhesive strips) and adhesive removal time.
Gait Analysis/CatWalk test: Gait analysis is performed using the Catwalk automated gait analysis system ([redacted]), which represents a sensitive tool to assess animal gait and locomotion. The mouse voluntarily traverses a glass walkway, and its footprints are captured and quantified with camera and specialized software to generate numerous parameters for qualitative and quantitative analysis of individual footprints and gait. The animals are trained on the walkway, until at least 3 compliant (1-5 sec duration, maximum deviation 60%) runs are successfully performed and recorded according to the manufacturers instructions. Animals are trained one week before and 2 days after dMCAO, and subsequently tested once a week at 7, 14 and 21 days after dMCAO .

Post-procedural Monitoring 6.14.1.9

ARF personnel routinely monitor animals daily but when invasive procedures are conducted, the PI is responsible for additional post-procedural monitoring.

Select ALL additional monitoring that will be conducted by the PI laboratory.
If severe adverse outcomes are expected attach a clinical scoring sheet that defines objective criteria for early removal (requires Veterinary consult).
*Check "NA" only for non-invasive studies that do not require additional post-procedural monitoring by the PI's laboratory.

- [x] Appetite
- [x] Body Weight Measures
- [x] Clinical observation
- [x] Intractable pain
- [ ] Tissue, Spontaneous Tumor, or Tumor Transplant Sequella
- [ ] NA - No invasive procedures are conducted that warrant additional observation by the PI laboratory.

6.14.1.9.1

Appetite

Monitoring Appetite 6.14.1.9.1.1

For some group housed species (e.g. rodents), appetite is not easy to directly evaluate.
Instead and when applicable, select body weight as an indirect measure of food consumption/appetite.
Provide assessment criteria, early endpoint criteria, and monitoring frequency (e.g. sid, bid, etc.) as well as duration that appetite will be monitored following the procedure(s) or attach a clinical scoring sheet above to define endpoint criteria and enter frequency of observation in the text below.

once a day, 7 days following surgery

6.14.1.9.2

Body Weight Measures

Monitoring Weight Loss **6.14.1.9.2.1**

Note: Weight loss >15-20% compared to baseline or age matched controls is routinely considered criteria for early removal.
Provide assessment criteria, early endpoint criteria, and monitoring frequency (e.g. daily, twice weekly, weekly, etc.) as well as duration that appetite will be monitored following the procedure(s) or attach a clinical scoring sheet above to define endpoint criteria and enter frequency of observation in the text below.

animal weight is monitored daily, for 21 days after dMCAO

6.14.1.9.3

Clinical observation

Monitoring Clinical Observations **6.14.1.9.3.1**

Evaluate abnormal conditions such as, decreased activity, hunched posture, shivering, piloerection, rapid or labored breathing, aversion to handling, ocular/nasal porphyrin staining, gaunt, dehydration, moribundity, etc.
Provide assessment criteria, early endpoint criteria, and monitoring frequency (e.g. sid, bid, tid, etc.) as well as duration that appetite will be monitored following the procedure(s) or attach a clinical scoring sheet above to define endpoint criteria and enter frequency of observation in the text below.

daily, for 7 days following surgery

6.14.1.9.4

Intractable pain

Monitoring Pain **6.14.1.9.4.1**

Observation for pain is required following procedures that pose a risk for significant pain or distress. When pain, distress or significant alteration of physiology is expected, it is recommended that a *numerical clinical scoring sheet be used to delineate the end point or point for intervention and this should have been attached above.
Provide assessment criteria, early endpoint criteria, and monitoring frequency (e.g. sid, bid, tid, q6h, etc.) as well as duration that appetite will be monitored following the procedure(s) or attach a clinical scoring sheet above to define endpoint criteria and enter frequency of observation in the text below.

daily, 4 days following surgery

Animal Disposition Methods **6.14.1.10**

Select all animal disposition methods that apply to this species.

- [x] Euthanasia
- [ ] Transfer to Another Approved Protocol
- [x] Transfer to ARF for Disposition
- [ ] Other

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6.1 4 .1.
10.1

Euthanasia

Euthanasia Table 6.14.1.10.
1.1

Fill in the information below.
Click the green plus icon to the right to add rows to the table.

*In addition, you may attach an SOP if applicable by clicking on the paperclip icon to the right.

Primary Method	Secondary Method
isoflurane overdose	150 mg/kg pentobarbital solution

Euthanasia Description 6.14.1.10.
1.2

Be sure to describe both chemical (e.g. CO2, etc.) and mechanical (e.g. decapitation, etc.) means of euthanasia if both are used.

We use the "Recognition and Alleviation of Pain and Distress in Laboratory Animals" (Committee on Pain and Distress in laboratory Animals, Institute of laboratory Animal Resources, National Research Council, National Academy Press, Washington D.C. 1992), as the guide for identification of these signs and adheres the practices recommended therein. Mice used in these experiments are euthanized by isoflurane overdose or i.p. injection of 150 mg/kg pentobarbital solution.

AVMA Guidelines for Euthanasia 6.14.1.10.
1.3

Are euthanasia methods consistent with the current AVMA Guidelines?
See Appendix 1, p. 111 for methods that are "acceptable" and "acceptable with conditions" and Appendix 2, p. 112 for methods that are currently "unacceptable".

Procedures that are "acceptable with conditions" and "unacceptable" to the AVMA both require scientific justification.

Click on the link below for more guidance.

- [x] Acceptable
- [ ] Acceptable with Conditions (e.g. CO2, cervical dislocation, or decapitation without anesthesia)
- [ ] Unacceptable or Not Consistent with AVMA Guidelines

6.1 4 .1.
10.1.3.1

Acceptable

Disposition of Animal Carcasses 6.14.1.10.
1.4

Describe the final disposition of carcasses and/or tissues following euthanasia.

The carcases and tissue will be placed in biohazard bags by the research staff. Bagged animal carcasses and tissue are placed in the provided storage freezer in [redacted].

Assurance of Death Following Euthanasia **6.14.1.10.1.5**

Please select ALL that apply.
At a minimum, the first four methods on this list should be used to confirm death in all cases when performing euthanasia, regardless of the chemical method employed. However, if mechanical methods such as decapitation or exsanguination are used as a means of assuring death, other methods of assuring death are unnecessary to evaluate.

Euthanasia must be painless and FINAL, with no chance of recovery.

- [ ] Lack of Heartbeat for one minute
- [ ] Lack of Respiration for one minute
- [ ] Lack of Response to Stimulus (e.g. toe pinch)
- [ ] Blanching of the eyeball (pale globe, indicating no blood is flowing through the eye)
- [ ] Cervical Dislocation
- [x] Decapita ion
- [ ] Exsanguination or Tissue Harvest after death or while under anesthesia

6.14.1.10.1.5.6

Decapitation

6.14.1.10.3

Transfer to ARF for Disposition

Agents and Occupational Risks

Occupational Risks Associated with Animals or Tissues Prior to Research Manipulation **7.1**

Some naive animals or animal tissues pose intrinsic occupational risks to personnel since they may harbor naturally endogenous zoonoses or animals may pose threat of physical injury.
If applicable, please indicate any specific occupational risks associated with animals or animal tissues used under this project and indicate methods to be utilized that will minimize such risks.

N/A

Biological, Chemical or Radiological Agent Use **7.2**

Will you be using biological, chemical, or radiological agents of any kind (both hazardous and non-hazardous)?

- [x] Yes
- [ ] No

7.2.1

Yes

Agent Type **7.2.1.1**

Select all that apply.
Biological - bacterial, fungal, parasitic, rickettsial, viral, biological toxins, prions, nucleic acid, cell lines, and primary tissue
Chemical - carcinogens, mutagens, nanoparticles
Radiological - administration of radioisotopes or exposure to radiation
Click the links for more information:

- [x] Biological
- [x] Chemical
- [ ] Radiological

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7.2.1.1.1

Biological

Animal Biosafety Level 7.2.1.1.1.1

Select all that apply by clicking the green plus icon to the right.

ABSL-1

7.2.1.1.1.1.1

ABSL-1

IBC Protocol Approval 7.2.1.1.1.2

List the Institutional Biosafety Committee (IBC) protocol approval number/ID(s), if required.

[redacted]

7.2.1.1.2

Chemical

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Hazardous Agents 7.2.1.2

Please select all hazardous agents (biological, chemical, radiological) that you are going to use in animals under this protocol by clicking on the green plus icon on the right and complete the following:

Add methods for use under the "Comment" field.

Update or change predefined data as necessary to specify use and safety procedures.
You may click the green plus sign to choose that agent then select "OK".
If your agent is not listed here, please email HSC-OACC@salud.unm.edu and we will add it for you.

Formaldehyde

Comments	Parafonnaldehyde (4% in PBS, Affymetrix)) is used for the mouse tissue fixation. The MSDS is attached
Precautions	Fonnaldehyde is a colorless, strongsmelling gas. Laboratories typically use it as fonnalin, a methanol-stabilized water solution that contains 37%, 44% or 50% fonnaldehyde. It is also used as a solid polymer (parafonnaldehyde). OSHA has identified fonnaldehyde as a human carcinogen. Written standard operating procedures (SOP) & safety data sheets (SDS) must be readily available in every lab using fonnaldehyde. All UNM affiliated personnel using formaldehyde must follow these guidelines. How can I protect myself from formaldehyde? · Review the fonnaldehyde MSDS and use the exposure controls it references. · Use fonnaldehyde & its solutions in a fume hood. · PPE: Always wear splash goggles, laboratory coats, & impermeable gloves to prevent eye & skin contact. · Do not store formaldehyde & its solutions near strong oxidizers. · Formaldehyde reacts with hypochlorite to fonn the potent carcinogen, bis-chloromethyl ether.
Instructions	DON'T CLEAN A FORMALDEHYDE CONTAMINATED AREA WITH BLEACH! How do I dispose of formaldehyde waste? · Handle & dispose of fonnaldehyde as an Extremely Hazardous Waste. · Dispose of empty fonnaldehyde containers as hazardous waste. · Dispose of all tissues & carcasses that come into contact with fonnaldehyde as medical waste. · Waste container labeling, disposal request, and removal by SRS can be found on the UNM web site at: https://srs.unm.edu/chemical-safety/hazardous-waste-collection-request.php What do I do if there' s a spill or emergency? · Clean up small spills with absorbent material. Neutralize spill with sodium hydroxide, sodium sulfite, or Spill-X-FP. · For large spills, evacuate the area & contact 911 or [redacted]) Isolate & control access to spill zone. · For dennal & eye exposure, wash area immediately in eyewash/shower for at least 15 minutes.

Nucleic Acid (infectious and non-infectious)

Comments	Even though the microRNA inhibitor oligonucleotide is not hazardous, the safety cabinet will be used to make the working dilutions of the microRNA inhibitor. Safety equipment includes gloves, eye protection masks, and laboratory coats. Waste materials (included sacrificed previously injected animals) will be discarded in red plastic bags labeled with Biohazard signs; these bags will be discarded in the appropriate biomedical waste container in the [redacted].
Precautions	Biosafety practices, containment equipment and facilities are detennined by the IBC.
Instructions	Synthetic or Recombinant Nucleic Acid Molecules (infectious and non-infections fonns) The administration of recombinant nucleic acid molecules to animals requires IBC review and approval. The use of this agent (& all gene inserts) requires IBC review and approval. [redacted]

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Additional Agents Not Listed Above 7.2.1.3

Attachments: Exiqon_Page_2.jpg Paraformaldehyde.pdf EdU.pdf Oligonucleotide MSDS (USA) copy.pdf

List any and all agents that were not mentioned above (including non-hazardous agents) and include the information below if applicable:
Description of the agent to include agent classification and risk group (e.g. ABSL2; carcinogen; chemical hazard)

Precautions and special handling

Containment and decontamination methods

*Attach MSDS and/or SOP if available by clicking on the paperclip icon to the right.

Edu Injections:
EdU (5-ethynyl-2' -deoxyuridine) is a nucleoside analog to thymidine and is incorporated into DNA during active DNA synthesis. In contrast to BrdU assays, the EdU-Click Assays are not antibody based and therefore do not require DNA denaturation for detection of the incorporated nucleoside. In addition EdU is suggested to be a safe alternative to BrdU, used before. The safety procedures will be performed according to the MSDS (attached.)
Literature:
A chemical method for fast and sensitive detection of DNA synthesis in vivo. Adrian Salic, [redacted]. PNAS, 105 (7), 2415-2420 (2008)

In vivo microRNA inhibitor delivery. Please see IBC approval file and 2 additional files:
In vivo delivery of the miRNA inhibitors will be performed in the [redacted] under ABSL-1 conditions.

Distal middle cerebral artery occlusion (dMCAO) will be used as the mouse experimental stroke model. Injections of specific anti-miR-155 LNA inhibitor or control inhibitor (scrambled oligonucleotide) from [redacted] will be initiated at 48 hours after dMCAO and performed for 3 consecutive days. Oligonucleotides will be introduced via mouse lateral tail vein; the dose will be 10 mg/kg in saline, total injected volume 100 µl. The dose and frequency of injections is based on the manufacturer's recommendations. Inhibitors will be injected with the 1 mL disposable insulin syringe. All disposables, including the absorptive pad and protective clothing, will be disposed of as hazardous biological waste. Mice will be kept in the [redacted], under standard housing conditions (two mice per cage). The animals will be sacrificed at 7, 14 and 21 days after the injections.

All [redacted] microRNA knockdown oligonucleotides have the animal grade purity (see attached files). In vivo inhibition of miR-155, has been performed by other authors [1-4]. No adverse effect of the inhibition was reported. Moreover, specific inhibition of this microRNA is accompanied by reduced inflammation, which makes it a promising therapeutic target in pro-inflammatory conditions. Studies on miR-155-defficient mice demonstrated reduction of BBB leakage/breakdown in the animal models of neuroinflammation. miR-155-/- mice also exhibit accelerated wound healing process, thus demonstrating and increased regeneration capacity. Please see attached MSDS file.
References:
[redacted]

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Person Responsible for Biological, Chemical, or Radiological Hazards 7.2.1.4

Give the name of the person(s) in the lab who is/are responsible for the safe handling of hazards and what they are responsible for doing (SDS, training, handling, bioinventory, radiological survey, etc.).
Click the green plus icon to the right to add rows to the table.

Name	Email	Phone	Hazard	Responsibilities
[redacted]	[redacted]	[redacted]	microRNA inhibitor	safe handling, MSDS, training, bioinventory

Waste Management 7.2.1.5

Describe your waste management practices (for each agent if different) including any special handling or disposal of cages, bedding, carcasses, waste stream, etc.

All disposables, including the absorptive pad and protective clothing, will be disposed of as hazardous biological waste.

Assurance Statement

PI Assurances 8.1

Read and select all. The PI must agree to all provisions contained herein. Once selected and saved, this program will require an electronic signature by the PI that will signify the Principal Investigator's agreement with the following conditions and assurances.
The PI must be logged in and must save the checkboxes in order to generate the login that creates the e-signature. No one else may sign.

- ☑ Animals are essential for this project and the study does not UNNECESSARILY duplicate previous experiments.
- ☑ The minimum number of animals will be used to support the goals of this study.
- ☑ All procedures are conducted in a manner to minimize discomfort, distress and pain. Any unanticipated pain or distress, morbidity or mortality will be reported to the attending veterinarian and/or the IACUC.
- ☑ All personnel par icipating in animal activities on this protocol are adequately trained in the procedures in which hey are involved.
- ☑ All personnel are aware of ethical responsibilities associated with animal research activities and procedures for repor ing animal welfare concerns.
- ☑ The PI and all personnel associated with this study will follow procedures under the approved protocol and comply with all pertinent institutional, state and federal rules regarding the use of animals in research, testing or education.
- ☑ I understand that if this protocol expires, all animal work under the protocol must cease until a replacement protocol is approved and all remaining animals must be transferred to a holding protocol.
- ☑ All individuals associated with this protocol that will have contact with live animals or with animal tissues or body fluids have been informed of the requirement for participation in the Institution's Employee Occupational Health and Safety Program.

8.1.1

Animals are essential for this project and the study does not UNNECESSARILY duplicate previous experiments.

8.1.2

The minimum number of animals will be used to support the goals of this study.

8.1.3

All procedures are conducted in a manner to minimize discomfort, distress and pain. Any unanticipated pain or distress, morbidity or mortality will be reported to the attending veterinarian and/or the IACUC.

8.1.4

All personnel participating in animal activities on this protocol are adequately trained in the procedures in which they are involved.

8.1.5

All personnel are aware of ethical respons bilities associated with animal research activities and procedures for reporting animal welfare concerns.

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8.1.6

The PI and all personnel associated with this study will follow procedures under the approved protocol and comply with all pertinent institutional, state and federal rules regarding the use of animals in research, testing or education.

8.1.7

I understand that if this protocol expires, all animal work under the protocol must cease until a replacement protocol is approved and all remaining animals must be transferred to a holding protocol.

8.1.8

All individuals associated with this protocol that will have contact with live animals or with animal tissues or body fluids have been informed of the requirement for participation in the Institution's Employee Occupational Health and Safety Program.

Animal Numbers Experimental Design (Mouse d MCAO and microRNA injections)

Group	Procedure	Group size (3 yr)	Strain	Pain Category
dMCAO injected D7	Injected with microRNA inhibitor and imaged at 7 days after dMCAO	20	C57BL/6	D
dMCAO injected D14	Injected with microRNA inhibitor and imaged at 14 days after dMCAO	20	C57BL/6	D
dMCAO injected D21	Injected with microRNA inhibitor and imaged at 21 days after dMCAO	20	C57BL/6	D
S/injected D14	Injected with microRNA inhibitor and imaged at 14 days after sham operation (where dMCAO is exposed but not occluded)	20	C57BL/6	D
dMCAO control inhibitor	Injected with control miRNA inhibitor and imaged at 14 days after dMCAO	20	C57BL/6	D