Addressing Rigor and Reproducibility in the Research Strategy (Significance & Approach)

MSTP Grant Basics 2021

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Exercise

Research Strategy examples:
- Did you like one better than the other?
- List 3 strategies that worked well
- List 3 aspects that did not work well
Lecture Question Set/Assignment

1. Provide an outline for the Approach section of a grant…
   — Take into consideration the NIH requirements for rigor and reproducibility.
   — Include the major headings that would go under Approach, plus one level of subheadings.

2. List three types of information that might be appropriate and/or necessary to include under “Rigor of proposed research” in the Approach section.

3. Provide an outline of subsections for the Significance section…
   — Take into consideration the NIH requirements for rigor and reproducibility.
   — Include the major headings that would go under Significance and, if applicable, one additional level of subheading.

In addition, Colin, please send me (also by March 6th) a list of 3 things you liked, and 3 things you did not like, about the examples of Approach sections that were assigned reading for last Wednesday’s session. (Please do not comment on Specific Aims pages, which were included only for reference.)
NIH definition of Scientific Rigor (2018)…

• the strict application of the scientific method
• to ensure unbiased and well-controlled
  — experimental design
  — methodology
  — analysis
  — interpretation and
  — reporting
• of results

Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

Research Training Plan:
1) Specific Aims page, no > 1 page (include 2–4 aims)
2) Research Strategy, no > 6 pages
   a. Significance
   b. Approach
Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

Research Training Plan:
1) Specific Aims page, no > 1 page (include 2–4 aims)
2) Research Strategy, no > 6 pages
   a) Significance: Describe…
      • the importance of the problem or critical barrier to progress that the proposed project addresses.
      • the strengths and weaknesses in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project.
      • how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.
      • how the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be changed if the proposed aims are achieved.
   b) Approach

Green text: relevant to Rigor & Reproducibility

Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

a) Significance

b) Approach: Describe…
   • The overall strategy, methodology, and analyses to be used…
   • Potential problems, alternative strategies, and benchmarks for success
   • If the project is in the early stages of development, any strategy to establish feasibility/address management of any high risk aspects
   • How relevant biological variables, such as sex, are factored into research designs/analyses for studies in vertebrate animals/humans
   • Any procedures/situations/materials that may be hazardous to personnel and the precautions to be exercised
   • If research on Human Embryonic Stem Cells (hESCs) is proposed but an approved cell line from the NIH hESC Registry cannot be chosen, strong justification for why
   • If you are proposing to gain clinical trial research experience (i.e., you will not be leading an independent clinical trial), your role on the clinical trial

Blue text: new/relevant to Rigor & Reproducibility
Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

a) Significance

b) Approach: Describe…

• The overall strategy, methodology, and analyses to be used, including:
  • plans to address weaknesses in rigor of prior research that serves as the key support for the proposed project
  • experimental design and methods proposed; how they will achieve robust and unbiased results
  • how the data will be collected, analyzed, and interpreted, and any resource sharing plans, as appropriate.
  • methods for analysis and sample size determination, as appropriate

• Potential problems, alternative strategies, and benchmarks for success

• If the project is in the early stages of development, any strategy to establish feasibility/address management of any high risk aspects

• How relevant biological variables, such as sex, are factored into research designs/analyses for studies in vertebrate animals/humans.

Rigor of prior research – Instructions

A careful assessment of the rigor of the prior research that serves as the key support for a proposed project will help applicants identify any weaknesses or gaps in the line of research.

• In Significance section:
  Describe the strengths and weaknesses in the rigor of the prior research (both published and unpublished) that serves as the key support for the proposed project.

• In Approach section:
  Describe plans to address weaknesses in the rigor of the prior research that serves as the key support for the proposed project.
Rigor and Reproducibility in NIH Grants

NIH Instructions
Fellowship Research Strategy
Rigor and Reproducibility

Addressing
Rigor & Reproducibility

Wording for Other Sections
Examples of what NIH Likes

Our grant writing templates…

https://medicine.uiowa.edu/sercc/resources/writing-grants
Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

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1) Specific Aims page, no > 1 page (include 2–4 aims)
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      • how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.
      • how the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be changed if the proposed aims are achieved.
   b) Approach

Significance section

• Importance of the problem and/or critical barriers to progress
• Scientific premise and rigor of the prior research *
• Significance of the expected research contribution
  – Impact of the project on scientific knowledge / technical capability / clinical practice
  – Impact of the project on the field

* The relevant literature: Strengths and weaknesses
  • Rigor of study design (e.g. statistical power, blinded analysis)
  • Incorporation of relevant biological variables (e.g. detail regarding sex)

Your preliminary data that contribute to scientific foundation of proposal.
Significance section

Limit to <1 page

- Importance of Problem
- Scientific Premise and Rigor of Prior Research
- Significance of Expected Research Contribution
  - impact on scientific knowledge
  - impact on the field

Keep preliminary data brief; leave details/figures to Approach subsection

1) Importance of the problem and/or critical barriers to progress
2) Scientific premise and rigor of the prior research (can organize overall or by aim)
   - Numerous studies have...
   - However, studies X and Y have important limitations...
   - In addition, the rigor of study Z was not sufficient in that...
   - To overcome these gaps in rigor, we will... [keep this general here]
   - Thus, our proposed studies will circumvent the limitations of... by...
3) Significance of the expected research contribution
   - Impact of the project on scientific knowledge / technical capability / clinical practice
   - Impact of the project on the field

Specifically mention limitations ... good lead-in for innovation
If there was a lack of rigor and it's possible to discuss diplomatically...

https://medicine.uiowa.edu/sercc/resources/writing-grants
Significance section

1) Importance of the problem and/or critical barriers to progress

2) Scientific premise of study and rigor of prior research
   • Numerous studies have…
   • However, the limitations of those studies are…
   • To overcome these gaps in rigor, we will…
   • Thus, our proposed studies will circumvent the limitations of… by …

3) Significance of the expected research contribution
   • Impact of the project on scientific knowledge / technical capability / clinical practice
   • Impact of the project on the field

Example of funding agency expectations

NIH Individual Predoctoral Kirchstein NRSA Fellowships

a) Significance

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     • how the data will be collected, analyzed, and interpreted, and any resource sharing plans, as appropriate.
     • methods for analysis and sample size determination, as appropriate
     • Potential problems, alternative strategies, and benchmarks for success
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     • How relevant biological variables, such as sex, are factored into research designs/analyses for studies in vertebrate animals/humans.
Approach section

Approach

- Issues related to rigor and reproducibility
  - Addressing weaknesses in rigor of prior research
  - Strategies to ensure rigor of proposed research
  - Consideration of biological variables including sex

- Aim x (for each aim)
  - Title of Specific Aim
  - Introduction/rationale paragraph
  - Justification and Feasibility paragraph
    (including background and preliminary data)
  - Research Design paragraphs
  - Expected Outcomes paragraph
  - Potential Problems and Alternative Strategies paragraph

- Timeline and Benchmarks for success
- Future Directions

Research Design Paragraphs:
- Approach to be used
- Overview of methods used
- Essential minor/major equipment
- Detailed expectations
- How results will be interpreted
Approach section

Approach

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2. Relevant biological variables including sex

- Sex (equal numbers of each; impact on results; separate analysis of effects; karyotype of cell lines)
- Weight, age, health status, body mass index, underlying comorbid conditions...

1. Rigor of proposed research → robust, unbiased results (discuss any of the categories below that apply)
  - Randomization protocol for sample groups, inclusion/exclusion criteria
  - Blinded data recording and analysis
  - Controls and replicates needed
  - Sample-size estimation/power analysis (critical for studies using human subjects and higher vertebrates)
  - Principles of good laboratory practice
  - Essential reagents and their authentication
  - Statistical analyses to be used
  - Controls and replicates needed

Separate paragraphs or combined

Approach section

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Future Directions
**Example of funding agency expectations**

NIH Individual Predoctoral Kirchstein NRSA Fellowships

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b) Approach: Describe…

- The overall strategy, methodology, and analyses to be used, including:
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**Approach section**

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Approach section

Example of Strategies to Ensure Rigor (from our authors)

R37 Renewal, scored in 2nd percentile – New subsection (after Aim 3)

Research Rigor and Transparency: Scientific rigor and reproducibility is maintained when opportunities for error are minimized through education of the team members about potential sources of error. To this end, the PI, staff, and students consult a Biostatistics and Research Design Core within the UI Institute for Clinical and Translational Sciences in the methodological planning of research protocols. This ensures robust statistical outcomes and post-experimental analysis of data. The PI and all associated personnel have also received NIH-mandated ethics training. All data will be reviewed by multiple team members to ensure its validity and to minimize operator biases; this occurs formally at twice weekly lab meetings, informally between trainees and the PI, and at the time of manuscript preparation, when the PI reviews all the raw data files. Morphometric analysis will be performed by blinded teams of students. Inbred C57BL6 strains will be used, with the exception of CF mice for which sibling CF and WT or heterozygous animals will be compared as previously described.9

Key points:
- Multiple approaches used to test each hypothesis.
- Multiple steps in process of data review and analysis ensure validity and minimize author bias.
- The rigor of the scientific approach is outstanding.
Examples of Rigor in Applications – posted by NIH 2016

- Excerpts from awarded applications reviewed under a pilot FOA for rigorous experimental design … this is only one part of updated instruction and review language.
- Selected based on high overall impact scores and positive reviewer comments specific to rigor.
- Provided to show how elements of rigor and transparency have been succinctly provided in applications; they may not represent all of the aspects/may still have room for improvement.
- May be updated as applications are reviewed and awarded under the revised rigor and transparency review.

Key points:
- Number of groups, allocation random, age, why that age.
- Dosage, number of doses administered
- Route of administration, contingency
- Group size, power
- Blinding, of whom
- Experience

Example 1:

**Aim 3**: Male and female mice will be randomly allocated to experimental groups at age 3 months. At this age the accumulation of CUG repeat RNA, sequestration of MBNL1, splicing defects, and myotonia are fully developed. The compound will be administered at 3 doses (25%, 50%, and 100% of the MTD) for 4 weeks, compared to vehicle-treated controls. IP administration will be used unless biodistribution studies indicate a clear preference for the IV route. A group size of n = 10 (5 males, 5 females) will provide 90% power to detect a 22% reduction of the CUG repeat RNA in quadriceps muscle by qRT-PCR (ANOVA, α set at 0.05). The treatment assignment will be blinded to investigators who participate in drug administration and endpoint analyses. This laboratory has previous experience with randomized allocation and blinded analysis using this mouse model [refs]. Their results showed good reproducibility when replicated by investigators in the pharmaceutical industry [ref].

Key points:
- Number of groups, allocation random, age, why that age.
- Dosage, number of doses administered
- Route of administration, contingency
- Group size, power
- Blinding, of whom
- Experience

Example 2:

**Aim 1**: Primary screen: In this high throughput screening assay, we combined the SMN promoter with exons 1-6 and an exon 7 splicing cassette in a single construct that should respond to compounds that increase SMN transcription, exon 7 inclusion, or potentially stabilize the SMN RNA or protein [refs]. The details of the assay and the SMN2-luciferase reporter HEK393 cell line have been extensively validated [refs]. Each point is run in triplicate, the compounds are tested on three separate occasions, and the results are averaged to give an EC50 with standard deviation. Secondary screen: … We analyze SMN protein levels by dose response in quantitative immunoblots with statistical analysis by one-way ANOVA with post-hoc analysis using Dunnett or Bonferroni, as appropriate.

**Aim 2**: Each set of compounds will include a blinded negative control compound that has been determined to be inactive and that is solubilized in the same manner as test compounds. Mice will be randomly assigned within a litter, and data will be collected and submitted to the PI. For compounds that demonstrate extended survival, the PI will be sure to have these tested in (the collaborators’) labs, and data will be merged and evaluated. To calculate the number of the experimental mice, we will perform an SSD sample size power analysis to ensure that the appropriately minimal number of mice is used in each experimental context. Typically for each compound in life span studies, we will need ~20 SMA animals in the treated group; ~20 SMA animals in the vehicle treated group; ~20 SMA animals in the untreated group. If we can administer the compound in aqueous solution without expedient, the vehicle and untreated groups might be combined, as these should have identical survival. Therefore, no more than 80 SMA animals will be needed per compound.
Examples of Rigor in Applications – posted by NIH 2016

Example 3:

Aim 2: Intensity signal data will be transformed into log values and then modeled by longitudinal methods (reference cited). Specifically, the composite difference in mean intensity signals over time between the bi-specific T cells vs. control groups is assumed to be 2.8 logs with a composite standard deviation of 2.2 logs. Furthermore, we will assume at least five repeated measurements per mouse after T cell infusion and a within-mouse intra-correlation coefficient equal to 0.50. Thus, a sample size of 10 mice per group will provide at least 80% power to detect the above difference between treated versus control group with a 5% significance level. Log-rank test will be used to compare the survival distribution between groups. VAS: Animal numbers are based on the requirement to perform each experiment (power and sample size calculations are described in the Research Strategy), which includes an independent experimental repeat.

Key points, Example 3:
• Methods for conversion of signal data and modeling
• Number of measurements and assumptions made for power analysis
• Statistical measures to be used
• Numbers of animals needed; to be determined independently for each experiment

Example 4:

Aim 1: Statistical considerations: In our preliminary studies consisting of this same cohort of DFUs (n=100) and utilizing 16S rRNA sequencing, we were able to detect dimensions of DFU microbiome, including microbial diversity, that were significantly associated with DFU outcomes. We therefore anticipate that the sample size will provide sufficient power to detect significant differences using metagenomic sequencing, as this is a more sensitive and less-biased assay of microbial identification and diversity.

Key points, Example 4:
• Statistical considerations based on preliminary data
• Anticipated power of sample size for new, more sensitive assay
• Statistical measures to be used

Descriptions of/Links to Biostatistics Resources

Rigor and Reproducibility
https://grants.nih.gov/reproducibility/index.htm

Scientific Editing and Research Communication Core

Biosciences Resources available to CCOM Researchers

https://medicine.uiowa.edu/sercc/resources/biostatistics-resources-available-ccom-researchers
Consideration of Sex as a Biological Variable (SABV)

**Reviewer Guidance to Evaluate Sex as a Biological Variable (SABV)**

**Main points**
- All research that uses sex as a biological variable will be factored into research designs, analysis, and reporting in vertebrate animal and human studies.
- Strain or sex should be stratified, randomized, and balanced, or other relevant considerations must be provided for applications proposing to study only one sex.
- The decision to use sex as a variable, when it is not necessarily part of the study, should not encourage the entire policy. See NOT-OD-15-102 for more information.

1. **Is the study focused exclusively animals or humans?**
   - Yes
   - No
   - No further consideration of sex or not considered a variable.

2. **Are both sexes included in the study?**
   - Yes
   - No

3. **Is the study designed to detect sex differences?**
   - Yes
   - No

4. **Are Acknowledgments as a strength in the critique and discussion and research outcomes?**
   - Yes
   - No
   - Not included in the study.

5. **Are the original datasets given to respond data and incorporating biomarkers?**
   - Yes
   - No

**Note:**
- For more information, see: https://grants.nih.gov/reproducibility/index.htm
- Rigor and Reproducibility I grants.nih.gov
- NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in vertebrate animal and human studies. Strong justification from the scientific literature, preliminary data, or other relevant considerations must be provided for applications proposing to study only one sex. NIH strongly encourages investigators to discuss these issues with NIH program staff prior to submission of applications.

Example of Consideration of SABV

"Recent" (2016) example including SABV – New subsection (before Aim 1)

**Methods to achieve robust and unbiased results:**
... and WT littermate controls were generated as described in Fig. 1. These lines were genotyped and cataloged across 10 backcrosses into the C57BL/6J strain. Only animals that are of the same genetic background and handled in the same way will be compared. Congenic Xxxx KO mice (B6.129P2-Xxxxx/J; stock #xxxx) were obtained from Jackson Laboratories. These mice had been backcrossed with C57BL/6J animals >30 generations. For cultures of dissociated PFC cells obtained from neonates, there is no reason to think that gender differences exist; hence male and female pups will be randomly allocated to experimental groups at P1. For the experiments involving [brain] slices from P30 animals, samples will be prepared from equal numbers of age-matched male and female animals and results will be tracked by gender. Each experiment will be performed in triplicate and repeated at least three times. Dose-response and time-course analyses will be conducted for each compound to ensure that the responses are maximal. We have extensive experience with blinded analysis, treatment paradigms, and group analyses generated using ANOVA followed by posthoc testing with Student’s t-test.
Rigor and Reproducibility in NIH Grants

NIH Instructions
Fellowship Research Strategy
Rigor and Reproducibility

Addressing
Rigor & Reproducibility

Wording for Other
Sections
Examples of what NIH Likes

Approach section

Approach

- Issues related to rigor and reproducibility
  - Addressing weaknesses in rigor of prior research
  - Strategies to ensure rigor of proposed research
  - Consideration of biological variables including sex

- Aim x (for each aim)
  - Title of Specific Aim
  - Introduction/rationale paragraph
  - Justification and Feasibility paragraph
    (including background and preliminary data)
  - Research Design paragraphs
  - Expected Outcomes paragraph
  - Potential Problems and Alternative Strategies paragraph

- Timeline and Benchmarks for success
- Future Directions
Introduction paragraph, formula:

• Justify why this aim needs to be performed / what aspect of the overall problem will be addressed (1–2 sentences only)

• Explicitly state the objective, e.g. "The objective of this aim is to…"

• Restate (verbatim) the working hypothesis from Specific Aims page, e.g.:
  "To attain the objective of this aim we will test the working hypothesis that …"

• State the overall strategy / approach for testing hypothesis (1–2 sentences)

• Provide rationale for work under this aim (i.e. what will become possible after this work is carried out)

• Summarize overall outcome and positive impact of this aim, at a general level

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Justification and Feasibility paragraph:

• Will potentially include two kinds of information

  • Justification of need — based on the literature
  • Evidence that you can do the work necessary to solve the problems you have framed — preliminary data

• If so, you need a good transition that bridges justification of need to evidence of feasibility, e.g.:

  These findings illustrate that … identifying x will be necessary to understand …, which will require knowledge of … . The following preliminary data support the feasibility of this approach in our hands.
Research design paragraphs:

- Write paragraphs related to research activities that will be undertaken to accomplish the objectives of that aim.
- In each paragraph, make a single conceptual point.
- Start each research activity (subaim) off with an interest-grabbing headline.

Aim 2.1. Determine which cells require the Mmd protein
Approach / methods overview / essential reagents / critical equipment / numbers of subjects/animals and how numbers were derived
statistical analysis to be used / controls / replicates / detailed expectations / how results will be interpreted / any major anticipated problems / time to complete

Aim 2.2. Identify the mechanism whereby TXS activity repositions Mmd
Approach / methods overview / essential reagents / critical equipment ...

Research design paragraphs:

- In writing research activities for each aim:
  - Emphasize concepts
  - Avoid anything tangential
- For methodologies:
  [as a student, you may need to include more detail than more senior researchers]
  - Reference any appropriate papers by your research team
  - If nobody on team has published with a certain methodology, include preliminary data on this
  - Where methods are repeated, refer to earlier descriptions of protocols
Approach section

Expected Outcomes paragraph (very important!):

• Purpose – Highlight expected return on the agency’s investment more explicitly than in summary or in introductory paragraph for the aim.

• In this paragraph you should:
  ▪ summarize expected outcomes for this aim (one per activity)
  ▪ convey how outcomes collectively achieve the objective of the aim
  ▪ underscore importance of this activity to:
    ◦ the field, of its own accord
    ◦ the overall objective of this aim
  ▪ mention any important caveats

Example language for this —

These experiments will provide the first analyses of .... Biochemical analyses will provide .... yielding a level of knowledge that has not been achieved in other systems. Combining this information with pharmacologic perturbations .... will yield insight into the function of ...., will also provide insight into .... Thus, the results will provide a foundation for attaining the overall objective of the proposal, i.e ....
Approach section

Potential Problems and Alternative Strategies paragraph:

- Identify problems that could arise but probably won’t.
- Include only the most important and probable, e.g.:
  - assays might not be sufficiently discriminating
  - critical reagents/patient samples might not be available
  - your working hypothesis might be proven invalid

- For each, identify:
  - the nature of the problem
  - reasons it is unlikely to arise
  - alternative approaches you would try if it were to arise

Example of language for this:

Regardless of our hypothesis, the experiments within this aim will … and whether …
Given that the experiments within this aim use well established and routine genetic tools
to determine … it is unlikely that the experimental techniques will fail. However, if …,
the interpretation of the results could be problematic. To overcome such a complication,
we would … by …. For instance, our preliminary data indicates that…. Thus we would
use … to identify which ….
Approach section

Timeline

- Comes after all of the Specific Aims
- **Purpose** — to outline timeframe needed to complete each subaim (as table or paragraph)
  - Grant applications are often rejected because overly ambitious or not ambitious enough.
  - Carefully thinking through and presenting a timeline can circumvent these problems.
  - If possible, include a schematic component

<table>
<thead>
<tr>
<th>Aims/Tasks</th>
<th>Year 01</th>
<th>Year 02</th>
<th>Year 03</th>
<th>Year 04</th>
<th>Year 05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aim 1: Characterize the function of...</td>
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<tr>
<td>1. Identify which</td>
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<td>2. Define why</td>
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<td>3. Examine the effects of...</td>
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<td>Aim 2: Identify the cellular source of...</td>
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<tr>
<td>1. Define effects</td>
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<td>Aim 3: Identify the downstream...</td>
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<tr>
<td>1. Characterize the...</td>
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Approach section

Future Directions

- **Purpose** — to summarize:
  - where you expect the science to be at end of the study
  - how results will complete next step in the continuum projected in long-term goals statement of Specific Aims page
  - what the next steps in the continuum will be, and why they are important

- Example of language for this:

  ... The knowledge gained will provide the foundation for ... In the future we plan to probe the mechanisms of ... by ... We will also expand the studies outlined here to examine whether ... Overall, such studies will help us attain our long-term goal of ... by ...
Approach section

EXAMPLE PROPOSAL 3
Risk Factors for Glaucoma: Uncovering Their Genetic Basis Using Inbred Mouse Strains

Complication: the selection of mice used in the populating arrays would become more tightly limited to mice representing only the extreme phenotypes — significantly high and low CEC density— which would increase the likelihood of identifying relevant loci. Another way to overcome this problem would be the replacement of one strain with a different inbred mouse strain of the same CEC phenotype. This would be possible because we initially populated many different strains for CEC density.

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<td>1.1: Develop cohorts of heterozygous F2 progeny</td>
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<tr>
<td>1.2: Phenotype cohorts for CEC density and CCI</td>
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<tr>
<td>1.3: Genes that influence CECs</td>
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Future Directions
The identification of genetic loci that influence CECs will greatly contribute to our understanding of the pathogenesis of glaucoma. An understanding of the genetic basis of CEC density together with the current era of measuring CEC density in the clinic setting, our ability to assess risk for disease, treat disease, and prevent vision loss associated with glaucoma will be greatly advanced. Furthermore, genetic manipulation of loci to influence CECs as well as pharmacological studies aimed at manipulating CEC density and function could also be pursued for new preventative measures and treatments for glaucoma and other CEC-related diseases. This early detection would enable us to identify high-risk individuals, begin preventative treatments well before the onset of disease begins, and ultimately give us the opportunity to significantly reduce the incidence of vision loss associated with glaucoma.

Approach section

expression by screening a subset of rats of ages 1, 2, and 3, testing the S/H and using western technology to detect the expression of p-ERK1/2, p-JNK, and p-c-JUN in the eye. The results show that ERK1/2 and JNK are activated in the lens and also in the retina, suggesting that these pathways may play a role in the regulation of lens growth and maturation. This is consistent with previous studies showing that ERK1/2 and JNK are involved in the regulation of cell proliferation and migration.

I tested the hypothesis that the two genes, p-ERK1/2 and p-JNK, are activated in the lens and retina, suggesting that these pathways may play a role in the regulation of lens growth and maturation. The results show that ERK1/2 and JNK are activated in the lens and retina, suggesting that these pathways may play a role in the regulation of lens growth and maturation. This is consistent with previous studies showing that ERK1/2 and JNK are involved in the regulation of cell proliferation and migration.

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Approach section

**Failure Studies**

My proposed studies will provide many answers about the movement of NGF in SARS physiology and dysfunction. It is undeniable that many more questions will arise after completion of the existing body of work. I have already made mutant NGF constructs that are likely resistant to Calmodulin phosphorylation. While outside of the scope of my proposal, these constructs will help lay the foundation for future studies on calmodulin-NGF interactions.

While outside of the scope of my proposal, these xxx will...

In future...

Completion of this work will provide important information about...

…and will allow me to learn … on which I can build an independent scientific career.
Rigor and Reproducibility in NIH Grants

NIH Instructions
Fellowship Research Strategy
Rigor and Reproducibility

Addressing
Rigor & Reproducibility

Wording for Other Sections
Examples of what NIH Likes

NIH Example 1

Modern molecular techniques allow the researcher to assess the role of a specific gene in a disease process. The hypothesis is tested by analyzing敲击鼠 (knockout mice) and wild-type mice. The key conclusion is that knocking out the gene of interest results in a significant phenotype. This is consistent with previous studies and suggests a role for the gene in the disease process.

NIH Example 2

In order to address the reproducibility of the results, the following steps are taken:

1. Replicate experiments are performed to ensure consistency.
2. Data is analyzed using independent statistics to confirm results.
3. The experiment is repeated using different techniques to verify findings.

These steps demonstrate the rigor and reproducibility of the research.
NIH Example 1

In subthalamic nucleus, the substantia nigra pars reticulata (SNr) is a key brain region involved in regulating motor function. To better understand the role of SNr, researchers have been focusing on the subthalamic nucleus and its interactions with other brain regions.

**Graphical Abstract**

- **Title:** Subthalamic Nucleus and its Role in Motor Regulation
- **Authors:** Jane Doe, John Smith
- **Keywords:** Subthalamic Nucleus, Motor Function, Parkinson's Disease

**Introduction**

The subthalamic nucleus (STN) is a deep brain structure that plays a crucial role in the regulation of movement. Its dysfunction is associated with various neurological disorders, including Parkinson's disease. The STN is part of the basal ganglia circuit, which is involved in motor control and coordination. Understanding the STN's role in motor function is essential for developing effective treatments for movement disorders.

**Materials and Methods**

Researchers used a combination of electrophysiological recordings and computational models to investigate the STN's activity in different motor states. They recorded electrical activity from the STN in awake behaving animals and performed detailed analyses of the data.

**Results**

The results showed that the STN activity is highly correlated with the different phases of the movement cycle. The researchers found that the STN activity changes in response to motor commands, indicating its involvement in the execution of movement.

**Discussion**

The findings suggest that the STN's role in motor function is more complex than previously thought. The STN's activity is not just a correlate of movement, but it actively contributes to the coordination of motor actions. Understanding these mechanisms could lead to new therapeutic strategies for movement disorders.

**Conclusion**

The study provides new insights into the role of the subthalamic nucleus in motor function. Further research is needed to fully understand the mechanisms underlying the STN's role in movement disorders and to develop targeted therapeutic strategies.

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**References**


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