Candidacy Exam Workshop

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Outline

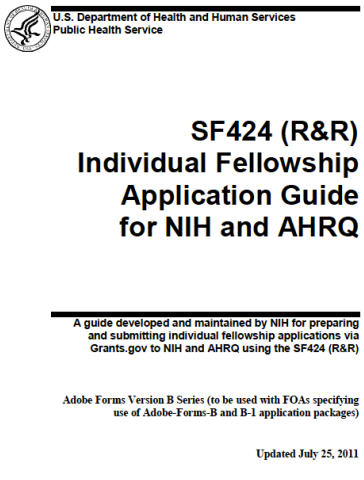
- Timeline
- Getting started
- Written proposal
- Tips
- The Candidacy Exam
- Submitting your document as an NRSA
- Thesis Committee

Timeline

- Due **February 1, 2013** to ARC (by e-mail to Josh Gold and Minghong Ma)
  - Proposal letter, including:
    - Tentative thesis title and advisor's name
    - Specific Aims page (limit one single-spaced page)
    - List of potential Candidacy Exam Committee (CEC) Chairs (2 names, and why)
    - List of potential CEC members and why (5–7 names in order of preference)
- Feedback from at least one ARC member by **February 15, 2013**
- Due **March 1, 2013**
  - Contact CEC, agree to serve
  - Schedule CEC meeting day, time and place
- Due **3 weeks** before your Exam
  - Final written document, all parts (not form pages)
- **10 days** after receiving document
  - CEC Chair and members provide feedback (in person and/or written)
- **7 days** to revise document if necessary
- **4 days** before Exam
  - Student must submit final, revised version to CEC Chair only
- Due **June 14, 2013**
  - All Candidacy Exams must be complete unless you have Josh's permission to delay

Getting started

- You will be preparing an application for a **Ruth L. Kirschstein National Research Service Award (NRSA)**
- Find the instructions: "Individual Fellowship Application Guide SF424."
- Read the instructions
- Follow the instructions
  - Page limit
  - Font size
  - Margins
- Ask 3rd year students for examples
- Set a time line for yourself



http://grants.nih.gov/grants/funding/424/index.htm#inst

Getting started

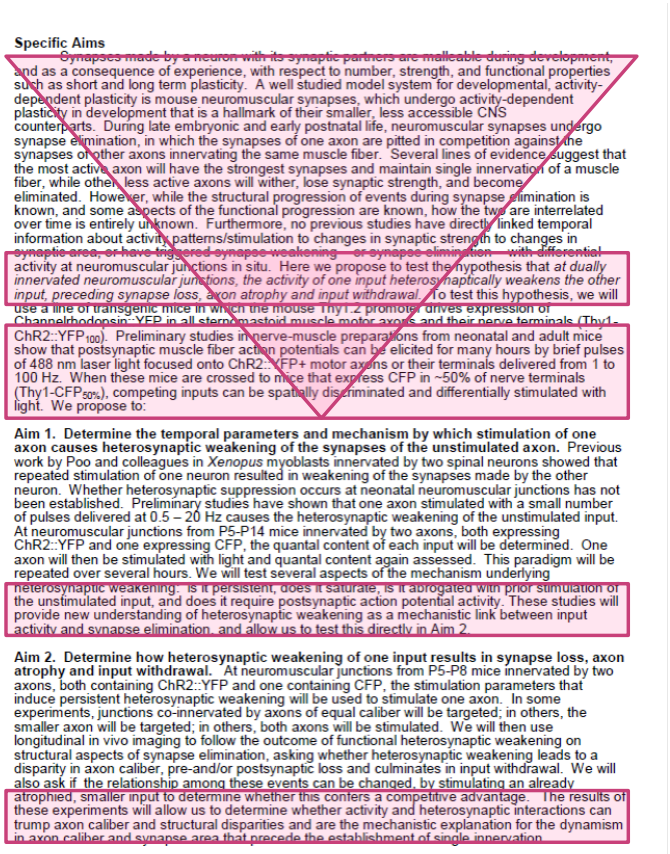
- Focus on a hypothesis that is central to your field
- Generate experiments to test that hypothesis
- Write these experiments in the form of Specific Aims
- Generate preliminary data to demonstrate the feasibility of the technical approaches
- Use preliminary data to demonstrate the likelihood of interesting outcomes that are interpretable and that advance the field

Written Proposal

- Cover page
- Project Summary/Abstract (1 paragraph)
- Specific Aims (1 page)
- Research Strategy (6 pages)
  - Significance
  - Approach (methods, preliminary data, timeline)
- Bibliography & References Cited

Tips: Specific Aims

- 1 page
- Inverted pyramid format
- Background paragraph that concludes with hypothesis (bolded, italics)
- 2 or 3 Aims, in paragraph form,
  - State goal
  - How you will test goal
  - Anticipated results
  - What results will mean for goal/hypothesis



Specific Aims

Synapses made by a neuron with its synaptic partners are malleable during development, and as a consequence of experience, with respect to number, strength, and functional properties such as short and long term plasticity. A well studied model system for developmental, activity-dependent plasticity is mouse neuromuscular synapses, which undergo activity-dependent plasticity in development that is a hallmark of their smaller, less accessible CNS counterparts. During late embryonic and early postnatal life, neuromuscular synapses undergo synapse elimination, in which the synapses of one axon are pitted in competition against the synapses of other axons innervating the same muscle fiber. Several lines of evidence suggest that the most active axon will have the strongest synapses and maintain single innervation of a muscle fiber, while other, less active axons will wither, lose synaptic strength, and become eliminated. However, while the structural progression of events during synapse elimination is known, and some aspects of the functional progression are known, how the two are interrelated over time is entirely unknown. Furthermore, no previous studies have directly linked temporal information about activity patterns/stimulation to changes in synaptic strength to changes in synaptic area, or have triggered synapse weakening – or synapse elimination – with differential activity at neuromuscular junctions in situ. Here we propose to test the hypothesis that *at dually innervated neuromuscular junctions, the activity of one input heterosynaptically weakens the other input, preceding synapse loss, axon atrophy and input withdrawal.* To test this hypothesis, we will use a line of transgenic mice in which the mouse Thy1.2 promoter drives expression of Channelrhodopsin-YFP in all sternomastoid muscle motor axons and their nerve terminals (Thy1-ChR2::YFP$_{100}$). Preliminary studies in nerve-muscle preparations from neonatal and adult mice show that postsynaptic muscle fiber action potentials can be elicited for many hours by brief pulses of 488 nm laser light focused onto ChR2::YFP+ motor axons or their terminals delivered from 1 to 100 Hz. When these mice are crossed to mice that express CFP in ~50% of nerve terminals (Thy1-CFP$_{50\%}$), competing inputs can be spatially discriminated and differentially stimulated with light. We propose to:

Aim 1. Determine the temporal parameters and mechanism by which stimulation of one axon causes heterosynaptic weakening of the synapses of the unstimulated axon. Previous work by Poo and colleagues in *Xenopus* myoblasts innervated by two spinal neurons showed that repeated stimulation of one neuron resulted in weakening of the synapses made by the other neuron. Whether heterosynaptic suppression occurs at neonatal neuromuscular junctions has not been established. Preliminary studies have shown that one axon stimulated with a small number of pulses delivered at 0.5 – 20 Hz causes the heterosynaptic weakening of the unstimulated input. At neuromuscular junctions from P5-P14 mice innervated by two axons, both expressing ChR2::YFP and one expressing CFP, the quantal content of each input will be determined. One axon will then be stimulated with light and quantal content again assessed. This paradigm will be repeated over several hours. We will test several aspects of the mechanism underlying heterosynaptic weakening: Is it persistent, does it saturate, is it abrogated with prior stimulation of the unstimulated input, and does it require postsynaptic action potential activity. These studies will provide new understanding of heterosynaptic weakening as a mechanistic link between input activity and synapse elimination, and allow us to test this directly in Aim 2.

Aim 2. Determine how heterosynaptic weakening of one input results in synapse loss, axon atrophy and input withdrawal. At neuromuscular junctions from P5-P8 mice innervated by two axons, both containing ChR2::YFP and one containing CFP, the stimulation parameters that induce persistent heterosynaptic weakening will be used to stimulate one axon. In some experiments, junctions co-innervated by axons of equal caliber will be targeted; in others, the smaller axon will be targeted; in others, both axons will be stimulated. We will then use longitudinal in vivo imaging to follow the outcome of functional heterosynaptic weakening on structural aspects of synapse elimination, asking whether heterosynaptic weakening leads to a disparity in axon caliber, pre-and/or postsynaptic loss and culminates in input withdrawal. We will also ask if the relationship among these events can be changed, by stimulating an already atrophied, smaller input to determine whether this confers a competitive advantage. The results of these experiments will allow us to determine whether activity and heterosynaptic interactions can trump axon caliber and structural disparities and are the mechanistic explanation for the dynamism in axon caliber and synapse area that precede the establishment of single innervation.

Tips: Specific Aims

- Generate a draft in consultation with your PI
- Anticipate generating many drafts
- Hardest part of grant to write
- Most important part of grant to read and for others to understand
- Must capture the Reviewer's interest – if not, you won't be successful in the peer review process

Tips: Significance

• Explain the importance of the problem or critical barrier to progress in the field that the proposed project addresses.

• Explain how the proposed project will improve scientific knowledge, technical capability, and/or clinical practice in one or more broad fields.

• Describe how the concepts, methods, technologies, treatments, services, or preventative interventions that drive this field will be changed if the proposed aims are achieved.

Tips: Approach

- Describe the overall strategy, methodology, and analyses to be used to accomplish the specific aims of the project. Include how the data will be collected, analyzed, and interpreted as well as any resource sharing plans as appropriate.
- Discuss potential problems, alternative strategies, and benchmarks for success anticipated to achieve the aims.
- If the project is in the early stages of development, describe any strategy to establish feasibility, and address the management of any high risk aspects of the proposed work.
- Point out any procedures, situations, or materials that may be hazardous to personnel and precautions to be exercised. A full discussion on the use of select agents should appear in Item 15, below.
- Include any courses that you plan to take to support the research training experience.

Tips: General

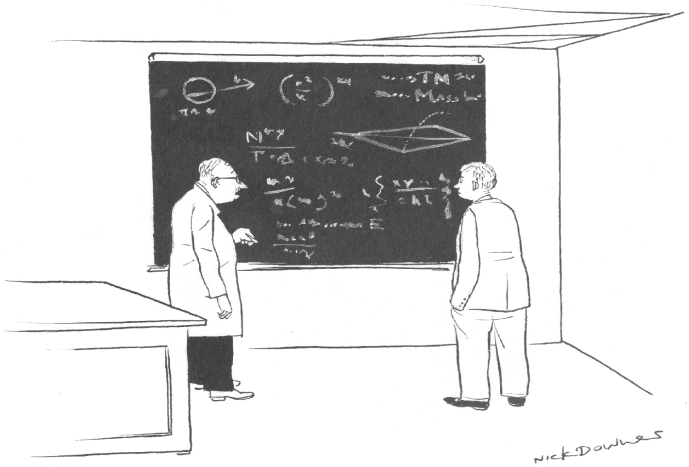
If an applicant has multiple Specific Aims, then the applicant may address Significance, Innovation and Approach for each Specific Aim individually, or may address Significance, Innovation and Approach for all of the Specific Aims collectively.

Tips: General

Preliminary Studies for New Applications.
For new applications, include information on preliminary studies, if any. Discuss the applicant's preliminary studies, data and/or experience pertinent to this application.

When applicable, provide a succinct account of published and unpublished results, indicating progress toward their achievement.

Tips: General



"In layman's terms? I'm afraid I don't know any layman's terms."

Tips: General

- **Consult the NGG Handbook**
- Focus on clarity
- Use resources – your advisor, faculty, labmates, classmates - for editing, reading, etc.
- Discuss NGG rules for Candidacy Exam with your Thesis Advisor
- Write, put aside, edit. Repeat. This takes time -- don't wait until the last minute
- Read document aloud to catch awkward writing, grammatical errors, etc.
- Spell check and format check

The Candidacy Exam

- Schedule 2.5 hours – exams typically take 2 hours plus some administrative time
- Bring your academic folder from Jane Hoshi and a printed copy of the Candidacy Exam Report form
- Prepare a ~30 minute research talk that is an overview of your Research Plan
- You step out of room for first ~10 minutes while Committee discusses your academic portfolio and Chair reports on document revisions, if needed
- Your Thesis Advisor is in room but may not participate in any aspect of the Exam, including asking or answering questions
- Anticipate questions throughout your talk
- Types of questions you can expect
  - Conceptual
  - Methodological
  - Interpretation
  - Significance
  - Breadth (typically at end, but not always)
- Student then steps out of room while Committee discusses evaluation

Outcomes

- **Pass**
- **Conditional Pass**
  - Document revisions necessary
  - Remediation necessary (coursework, re-examination, etc.)
- **Fail**

Submitting your document as an NRSA

- All eligible NGG students are required to submit a version of their proposal as an NIH NRSA or as a grant to another agency, within ~6–9 months of Exam.
  - NIH deadlines are August, December and April – but these change, so check
- Talk to Josh if there's some issue with this
- Grant submission is done through PennERA – contact BGS office for information. You must follow Penn deadlines for grant submission, typically at least 7 business days prior to NIH or other grant agency deadline
- NGG website → Resources → Wiki page with useful information, from Matt Nassar , Dan Denman and others. Contact Jane Hoshi for login and password. Note that some information may be out of date.

Submitting your document as an NRSA

Form Pages (check this for latest requirements):

- Cover Letter (including List of Referees)
- Biosketch
- Undergraduate and Graduate coursework, grades
- Previous Research Experience
- Goals
- Planned Activities
- Facilities and Other Resources
- Equipment
- Selection of Institution and Sponsor
- Respective Contributions
- Other Attachments
  - List of Referees (at least 3)
  - Sponsor's information (limit 6 pages)
- Letters of Recommendation

Thesis Committee

- Thesis Committee Chair and members must be approved by ARC Chair and NGG Chair (email)
- Thesis Committee must be formed by the end of Fall Semester, 2013
- First TC meeting must be held by the end of Spring Semester, 2013
- Meetings involve:
  - Preparing a Specific Aims page plus progress on each Aim (bullets): send to Committee no later than 3 days before meeting.
  - Preparing a 30 – 40 minute talk of background, Aims, data
- Your thesis proposal will evolve – that's expected
- Your first TC meeting will be the most comprehensive (2–3 hours)
- Subsequent TC meetings can be more focused (1–2 hours)
- TC meetings should be frequent: 1–2x / year