Changes to NIH grant applications:

• Changes to application guide instructions for preparing the **Research Strategy**. Three new required sections have to be incorporated into the 12 page limit of the Research Strategy. There is less room for other things in the Research Strategy. START EARLY!!!!

• A 4th required new section is for **Authentication of Key Biological and/or Chemical Resources.** This is in "Other Attachments" section of R&R Other Project Information component.

• **New instructions for peer review** include additional criteria for rigor and transparency (more on this below).

New required sections in Research Strategy:

1) The scientific premise forming the basis of the proposed research

2) Rigorous experimental design for robust and unbiased results

3) Consideration of relevant biological variables (example: sex representation in subject pool)

The scientific premise forming the basis of the proposed research

1) In the Significance section
Describe the scientific premise for the proposed project, including consideration of the strengths and weaknesses of published research or preliminary data crucial to the support of your application.

Rigorous experimental design for robust and unbiased results

In the Approach section

Describe the experimental design and methods proposed and how they will achieve robust and unbiased results.

Begley (2013) Six red flags for suspect work. *Nature.*

-Were experiments performed blinded?
-Were basic experiments repeated?
-Were all the results presented?
-Were there positive and negative controls?
-Were reagents validated?
-Were statistical tests appropriate?

Considerations for rigorous study design

- Pre-experiment power calculations (endpoint sensitivity, variability, effect size, desired level of confidence, definition and rationale for n).
- Controls to reduce bias in data collection
- Randomization
- Procedures to achieve blinding
- Data handling and analyses
- Positive and negative controls
- Thorough and transparent reporting
  - Steward and Balice-Gordon, (2014) Neuron, 84, 572-581.

Considerations for rigorous study design

- Experimental procedures to protect against un-recognized bias.
  - Is bias minimized through blinding, recoding, and systematic random sampling?
    - "Bias is unintentional and unconscious. It is defined broadly as the systematic erroneous association of some characteristic with a group in a way that distorts a comparison with another group…The process of addressing bias involves making everything equal during the design, conduct and interpretation of a study and reporting those steps in an explicit and transparent way" (Ransohoff and Gourlay, 2010).

- Random assignment to groups.
    - Allocation concealment
    - Prospective inclusion/exclusion

  - Blinding
    - Blinded outcome assessment
    - Separation of data collection and analysis. 3rd party data management
    - Re-coding data
    - Exceptions to blinding, and resulting interpretive caveats
- Biases due to outcome expectation: Is the guiding philosophy to "test" an hypothesis or "prove" an hypothesis?

  - Steward and Balice-Gordon, (2014) Neuron, 84, 572-581.

Considerations for rigorous study design

- Unbiased sampling:
  - Use defined protocols for sampling populations. Examples include well-developed methods for opinion surveys.
  - In studies of tissues and cells, use techniques for unbiased sampling that come from principles of “stereology.

Considerations for rigorous study design

- Data analysis:
  - Plan statistical analysis and get consultation BEFORE collecting data. An important part of good statistical analysis is in experiment execution.
  - Understand corrections for multiple comparisons. If you are collecting different data sets from a single group, this constitutes multiple comparisons even if the data are from different analyses.
  - Explain how you will avoid “testing to a foregone conclusion”.
    - *Red flag*: Collecting data, analyze as you go, and continue to increase “n” until differences are significant. This is the way pilot experiments are often done. The way to avoid the problem is to clearly distinguish between “pilot” experiments and the start of the definitive experiment.
  - Explain how you will avoid “p-hacking”.
    - Steward and Balice-Gordon, (2014) Neuron, 84, 572-581.

ADVICE: HAVE A SECTION WITH THE SUBHEAD:

"Rigorous experimental design"

Our studies follow best practices for pre-clinical experiments (Steward & Balice-Gordon 2014). Sample size determinations use data from our previous studies with the SCI models. For blinding, animals are assigned an ID number prior to treatment and assigned to groups based on a random number generator (Sharp et al 2014a). The order of surgical procedures is determined by a random number generator. Functional and anatomical assessments are carried out by individuals who are blind to treatment groups. For compilation, data are passed to an individual who is not involved in testing so that testers remain blind. Methods are transparently reported in our publications, including time of day and order of surgical procedures and animal testing, methods of random assignment to groups, exclusions and attrition.

ADVICE: HAVE A SECTION WITH THE SUBHEAD:

“Rigorous experimental design” (continued)

Exclusion criteria are predefined, and include: 1) surgical errors (spinal cord damage during laminectomy; out of range force parameters for the impactor; excessive bleeding, and out of range functional scores during the first week of post-operative testing); 2) development of pain symptoms or autophagia; 3) deteriorating health, excessive weight loss. #2 and 3 are animal welfare decisions based on consultations with veterinary staff. Sample size determinations include estimates of attrition based on our previous experience.

ADVICE: HAVE A SECTION WITH THE SUBHEAD:

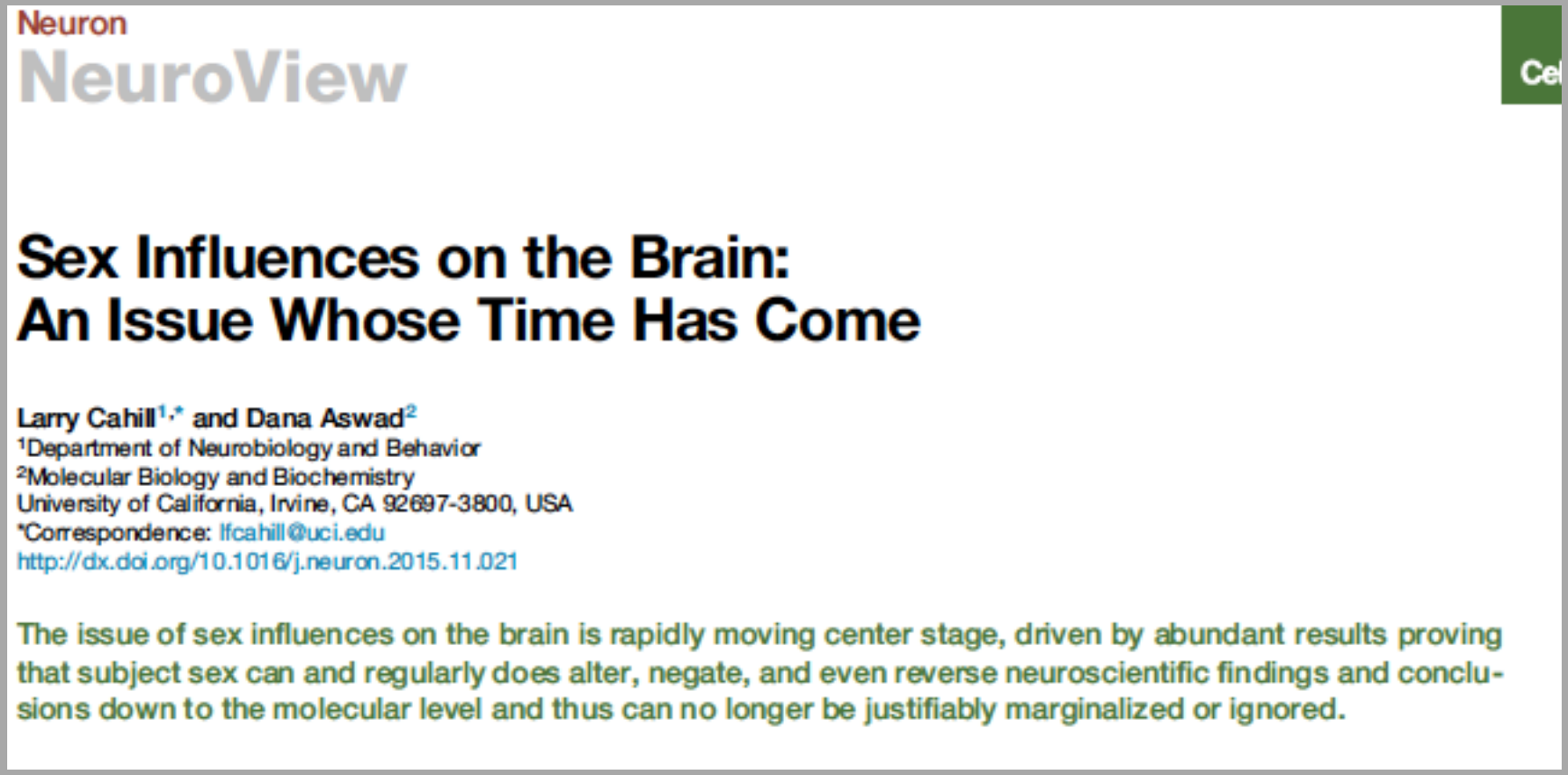
"Rigorous experimental design" (continued)

Statistical analyses are based on the data (homogeneity of variance, etc.) and always correct for multiple comparisons (Bonferroni type). We avoid *p-hacking* (multiple analyses in search of statistical significance). To reveal relationships, distributions, and data clustering, we plot individual animal data points rather than just means and SD and quantitatively assess relationships between measures by regression analyses, for example, functional vs. anatomical variables (Sharp et al 2014b). Interim statistical analyses are pre-planned to terminate experiments where attrition or variability diminish power. If there are trends that don't reach statistical significance, experiments are repeated rather than *testing to a foregone conclusion* by adding subjects*;* results of repetitions are reported separately rather than combining data (Sharp et al 2013).

For assessments that generate multiple data sets, we pre-define primary outcome measures, report all analyses that were carried out including non-significant results. Key experiments are repeated (self-replication). Quantitative anatomical studies are done blind using coded slides.

New required third section: Consideration of relevant biological variables (example: sex representation in subject pool)

This is a new required section in the Research Strategy, but follows earlier mandates. The paper below may be a useful resource for references.



Neuron

NeuroView

Sex Influences on the Brain: An Issue Whose Time Has Come

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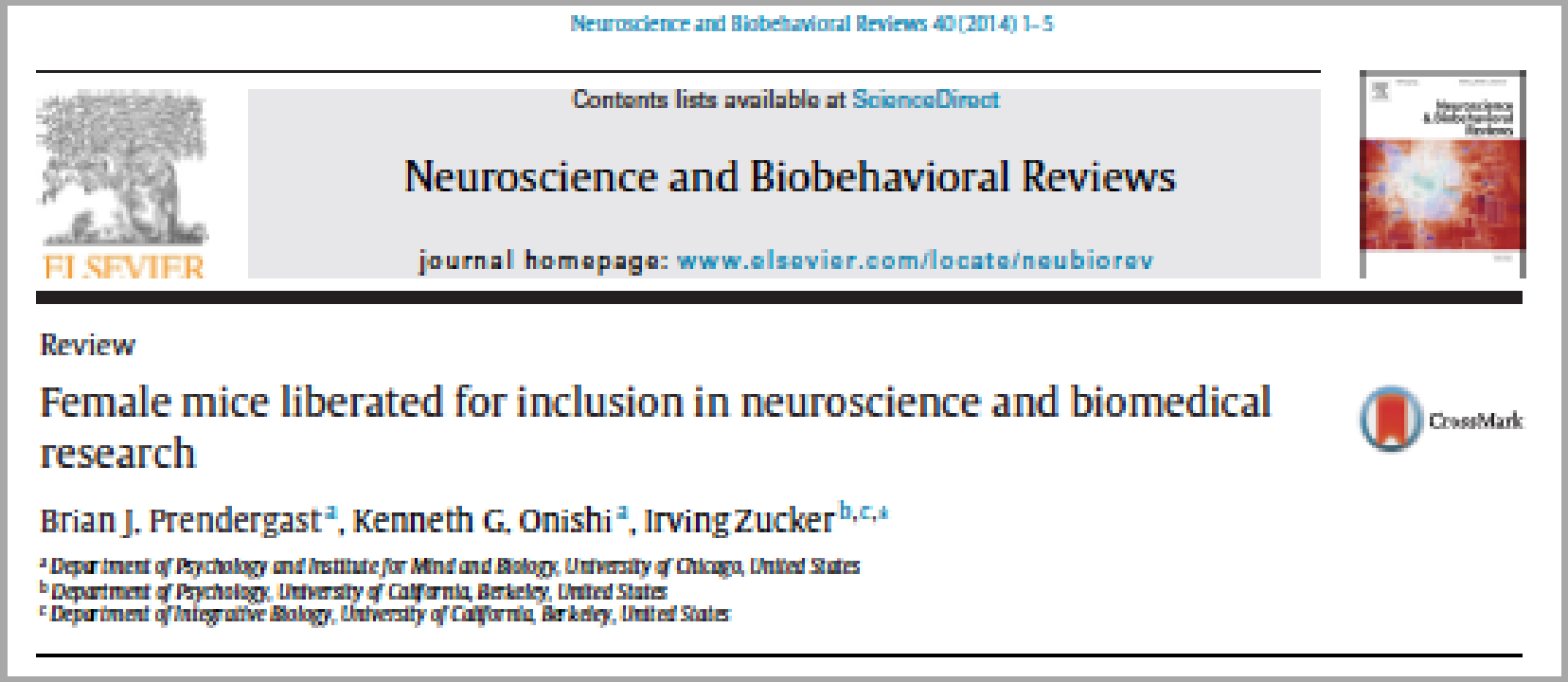
http://dx.doi.org/10.1016/j.neuron.2015.11.021

The issue of sex influences on the brain is rapidly moving center stage, driven by abundant results proving that subject sex can and regularly does alter, negate, and even reverse neuroscientific findings and conclusions down to the molecular level and thus can no longer be justifiably marginalized or ignored.

New required third section: Consideration of relevant biological variables (example: sex representation in subject pool)

Traditionally, male animals have been used for many studies because of concerns about variability over the estrous cycle. The paper below indicates that this may be less of a problem than expected and may be a useful reference for this point.



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Review

Female mice liberated for inclusion in neuroscience and biomedical research

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Authentication of Key Biological and/or Chemical Resources

Describe methods to ensure the identity and validity of key biological and/or chemical resources used in the proposed studies.

1) Cell lines

2) Antibodies

3) Drugs

4) Genetically modified animals. For transgenic animals carrying fluorescent marker for particular cell types, how will specificity be validated? Consider complications of genetic background.

This section is to be uploaded into the Other Attachments section and for now, **is not YET a criterion for scoring**.

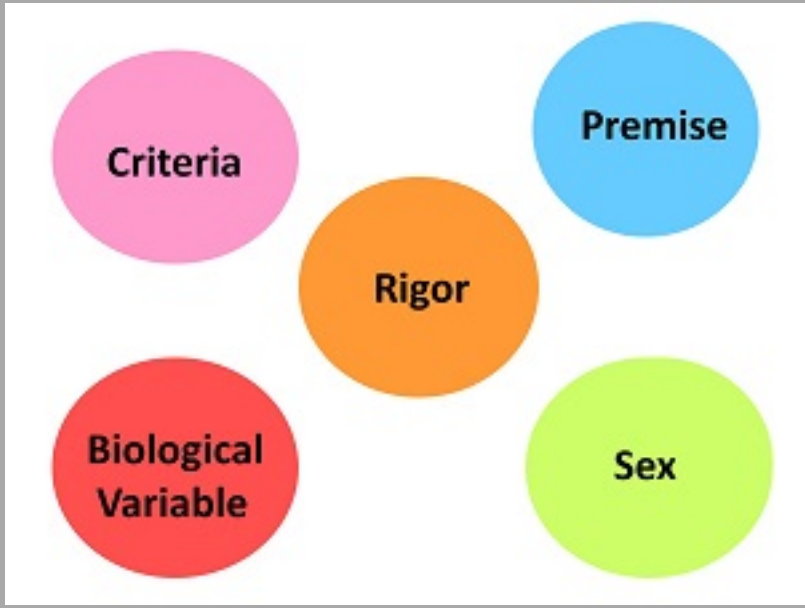
How will reviewers will react to and interpret the new guidelines?

- Enhancing rigor will likely require increasing research staff to enabling blinding, increasing “n” and repeating experiments, which will increase project cost and reduce the scope of what can be accomplished.

- How will steps to increase rigor be viewed given the current culture of peer review, which emphasizes innovation and eye-catching research?

http://www.csr.nih.gov/CSRPRP/2016/09/implementing-new-rigor-and-transparency-policies-in-review-lessons-learned/#more-579

Peer Review Notes: September 30, 2016 Implementing New Rigor and Transparency Policies in Review—Lessons Learned



After scientists at NIH in various fields and pharmaceutical companies raised serious concerns about the reproducibility of NIH research, NIH responded by launching multiple efforts to enhance the rigor and transparency of the research it funds. A big one involved changes in review criteria that we implemented in the last round of grant application reviews that involved 525 meetings and nearly 10,000 reviewers.

But work remains. Not everyone got the message.

NIH continues efforts to make it clear that it has elevated the degree of attention that must be paid to sex as a biological variable, and also to resource authentication.

Even those reviewers who have always thought carefully about scientific premise and rigor should reflect on the design and methodological considerations that are critical for work in their field.

Some reviewers thought about the new emphases but used old language in their critiques, which made it harder for SROs and program officers when dealing with large numbers of applications.

Premise" caused confusion.

NIH intended for reviewers to consider the scientific foundation of the proposed work. That is, reviewers should critically ask whether the studies or preliminary data leading to the proposed work are scientifically sound. Although this sounds like an obvious consideration, multiple studies, covering multiple fields of science show that scientists have often overestimated how replicable published work is—even when published in top journals.

Performing this review of premise can be intellectually demanding and many reviewers did a great job. Others confused "premise" with "scientific significance" or discussed whether the hypotheses of the study were reasonable. Significance refers to the importance of the study; premise refers to its scientific foundation. While a weak premise clearly undercuts the potential significance of a proposal, a strong premise (empirical foundation) does not necessarily make a study significant.

“Sex” was hard to talk about, sometimes. NIH expects that sex as a biological variable will be factored into research designs, analyses, and reporting in all vertebrate animal and human studies unless there is a compelling scientific argument for not doing so.

The impact of this policy varies considerably across different areas of science. Panels were challenged at times to really sort through—

What is convention, and what is good science?
What is adequate incorporation of sex in study design?
Can inclusion of both sexes actually reduce scientific rigor, for example by increasing physiologic variability?
If a disease affects one sex predominantly but not exclusively, is that sufficient justification for single sex studies?
What should investigators do with sex specific data that is not sufficient to investigate sex differences beyond reporting it?