Practical solutions for including sex as a biological variable (SABV) in preclinical neuropsychopharmacological research

DRIVERS Journal Club

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Why SABV Matters

- Traditional research in neuroscience, pharmacology,and behavioral studies are mostly conducted on male subjects
- Female exclusion is often justified by 'hormonal variability.'
- Findings generalized to both sexes, leading to biased outcomes
- historical resistance to include female-derived cells in *in vitro* studies, leading to a predominance of male-derived cell lines.
- Underrepresentation of females leads to
  - **poor drug efficacy**
  - **poor reproducibility**
  - **more adverse drug reactions in women**

Consequence: Over the past decades we missed biological insights in the sex-specificity of the most prevalent diseases (e.g., depression, Alzheimer's, Parkinson's, and cardiovascular diseases).

Discussion Questions

- Why has SABV been ignored for so long?
- What challenges do we face in implementing SABV?

Policy Shifts Towards SABV

- NIH, European Commission, CIHR require sex inclusion.
  - more grant applications address now SABV considerations (CIHR 48%)
  - researchers debating whether it is scientifically justified or politically motivated.
  - Concerns: increased costs, logistical complexities, and the potential need for larger sample sizes

J Womens Health (Larchmt) 2021 Mar 4;30(3):348–354.
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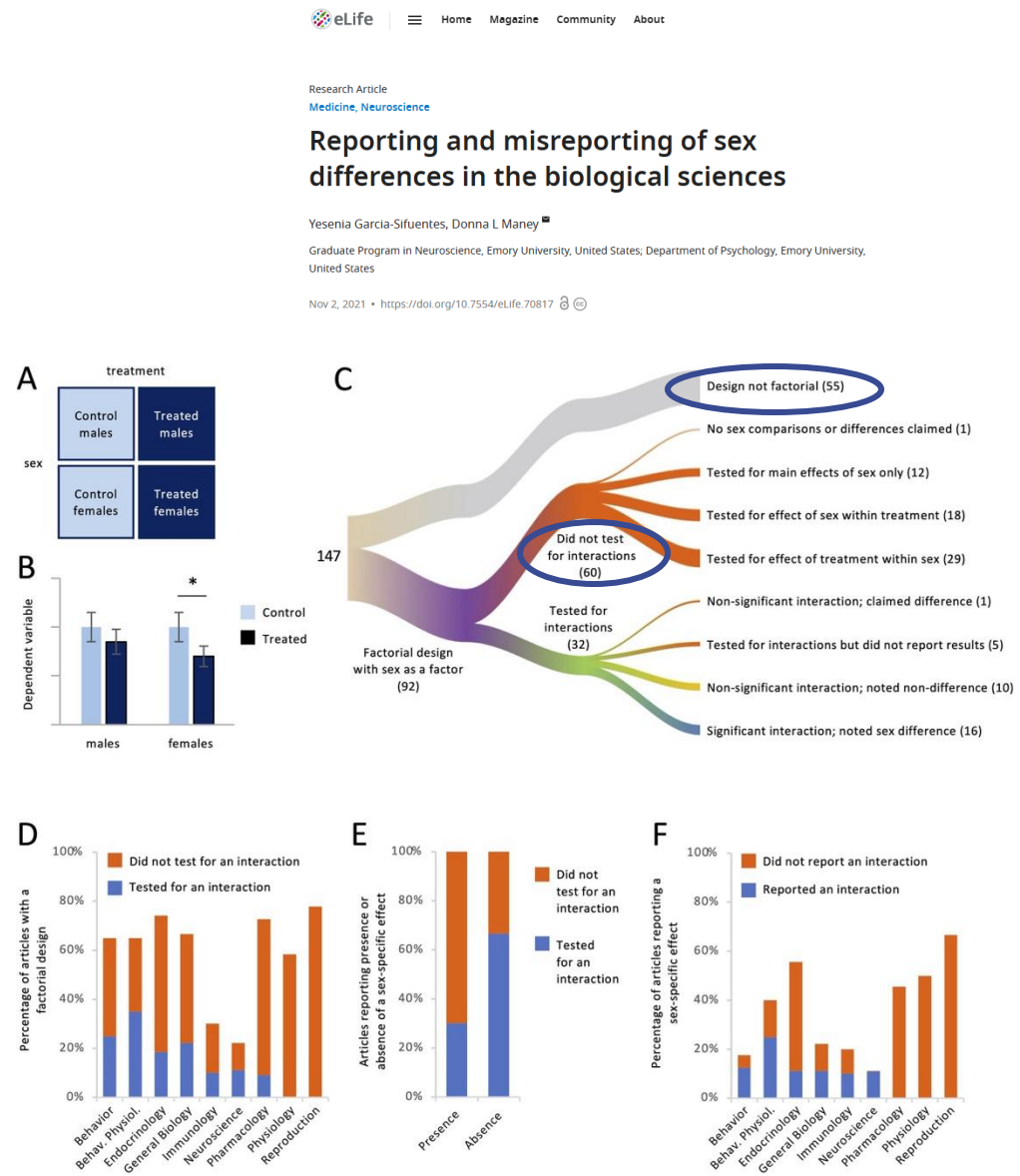
- Journals now demand reporting of sex differences.
  - Nature journals (2022): require authors to disclose whether and how sex and gender were considered in study design, data analysis, and interpretation.
  - APS journals (2012): required authors to report the sex of animals and cells used in studies
  - JNR (2017): mandated the consideration of sex as a biological variable in ms

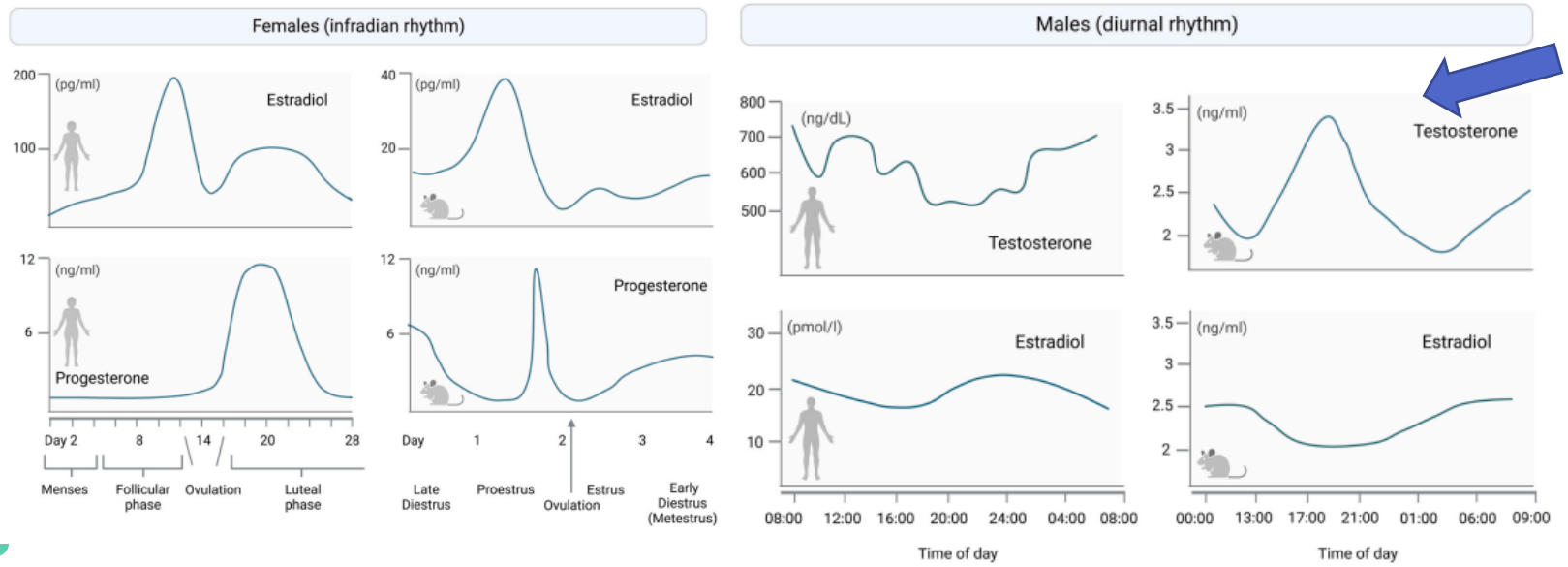
Challenges remain despite policy changes even when both sexes are included

- a significant number lacked rigorous statistical analysis to support these SABV claims, leading to potential overreporting or misinterpretation of sex differences.
- This underscores the need for continued education and policy enforcement to ensure accurate and meaningful integration of sex and gender considerations in research.



Common Myths About SABV

- Myth 1: Including females doubles sample sizes → Fact: Factorial designs reduce the need.
- Myth 2: Hormonal cycles make data too variable → Fact: Variability is equal in both sexes.



Tracking sex hormones in female rodents → easier in females than males
When relevant: Swab, lavage, or blood samples.

Behavioral Testing – Key Considerations

- male and female subjects may employ different strategies when subjected to the same behavioral tasks.
- Sex-specific behaviors exist (e.g., females use darting in fear response, whereas males may display passive behaviors such as freezing).
- Equipment should accommodate size/weight differences.
- Environmental & social factors influence behavior (housing conditions, handling by researchers, the presence of olfactory cues)
- Selection of behavioral tasks that are equally relevant and motivating for both sexes

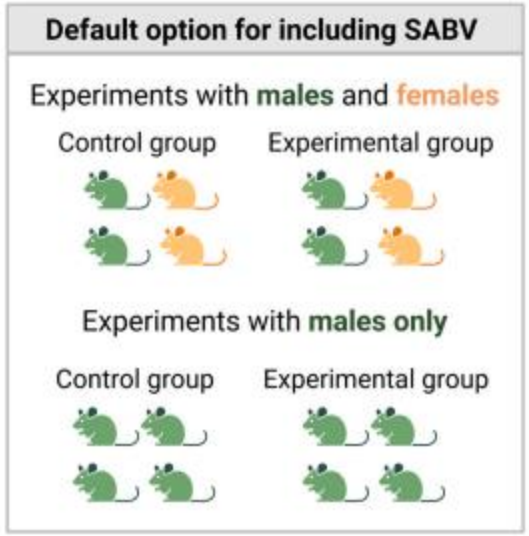
Lifespan Aspects

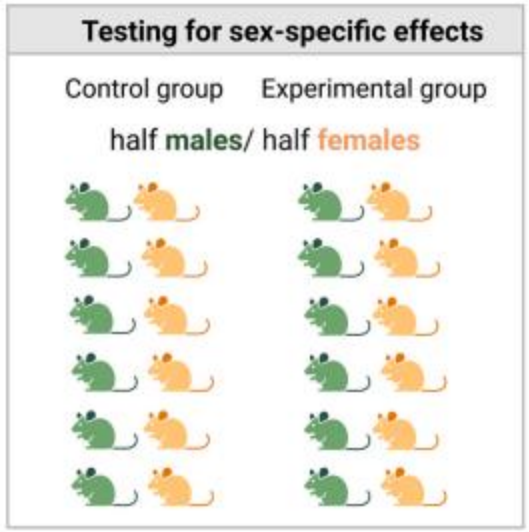
- Sex hormornal changes in rodents and human not always parallel
- Translatability of such studies
- Stress → pregnancy → neuronal cell cultures are prepared from“stress-effected“ embryos
- Pooling data from animals across age ranges (pre-puberty /puberty) can lead to false result
- Always include appropriate control groups

In vitro sex-specificity

- Sexual Dimorphism in Cell Population
- Challenges with Cell Sources:
  - **Immortalized Cell Lines:** from a single donor of unspecified sex; may not be representative, even when male and female versions exist
  - **Primary Cells:** more accurate representation, primary cells present challenges in availability and variability. Obtaining sex-matched primary cells requires careful donor selection and can be resource-intensive.
- Consideration of external factors (media composition, materials) → can influence outcome
- Reporting: Cell lines need to be labeled for sex.
- Drugs have different pharmacokinetics (PK) and pharmacodynamics (PD) in men vs. women.

Statistical design, analysis and reporting





- **Statistical Analysis Strategies:**
- **Factorial Design:** Implementing a factorial design allows researchers to assess the main effects of sex and treatment, as well as their interaction. This approach provides a comprehensive understanding of how treatments may differentially affect males and females.
- **Power Analysis:** Conducting a priori power analyses is recommended to determine the appropriate sample size needed to detect sex differences, ensuring that studies are neither underpowered nor unnecessarily large.
- **Transparent reporting:** including both significant and non-significant findings
- **Avoid pooling data** from both sexes unless justified

SABV – drug and therapy development

- **Sex Differences in Pharmacokinetics and Pharmacodynamics** (drug absorption, distribution, metabolism) →Incorporating both male and female subjects in preclinical studies is essential

- **Clinical Trial Design**: inclusion of both sexes in clinical trials, with sufficient power to detect sex-based differences in drug response.

- **Regulatory Guidelines and Policies:** a need for adherence to NIH and EU guidelines on SABV considerations to improve the translational relevance of research findings.

- **Recommendations:**
  - factorial study designs to efficiently assess sex differences,
  - conducting thorough literature reviews to inform hypothesis generation,
  - fostering collaborations among researchers to share data and resources

Take-Home Messages

- Including SABV improves research rigor & translational value.
- Statistical adjustments make inclusion feasible without doubling samples.
- For certain preclinical tests: Check whether Sex-specific behaviors exist
- Assessing sex hormones in blood samples is viable option for monitoring
- Addressing SABV early saves time & improves clinical outcomes.
- Report SABV sufficiently and in a transparent manner

Thank You & Final Discussion

- Should funding agencies mandate equal sex representation?
- What can research institutions do ? Do they even play a role?
- How do SABV considerations impact precision medicine?
- Open floor for additional thoughts & experiences.