Commentary

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Consideration of Research Approaches in Systems Neurobiology

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Macroscopic oscillations, derived from the synchronous activity of neuronal ensembles, generate rhythmic brain electrophysiological patterns that are highly coupled to behavioral and cognitive states (1,2). These oscillations are a key indicator of the communication status between neurons that together form circuits that collectively make up neural networks. In our search to understand the mechanisms that underlie behavior or cognition, and in our pursuit of novel biomarkers of disorder pathology, researchers have long assessed how circuit/network function alterations may contribute to neurological disorder manifestation (3,4). Unfortunately, we have not been as successful as hoped, with inconsistencies in many of the findings. There are likely many reasons for this, but it is perhaps the way in which our research is generally performed that has hindered our progress. As demonstrated by Douton and Carelli (5) in the current issue of *Biological Psychiatry: Global Open Science*, there are certain considerations in study design and methodology that could improve replicability in systems neuroscience research to ultimately advance biomarker and drug target discovery. Some considerations are offered below and in Box 1.

Sex and Gender Considerations. Historically there has been a lack of research that identifies sex- or gender-specific mechanisms in neurodevelopmental, neurological, and neuropsychiatric disorders, despite established sex and gender differences in prognosis, diagnosis, symptomology, and treatment responses. Only in recent years has there been a push toward the inclusion of female subjects in preclinical, translational, and clinical research.

The study of sex and gender differences is not a new idea, and it is important to acknowledge the many amazing scientists that have spent decades doing this type of research. Many of us, however, are guilty of not including female subjects in our research at some point. Others have been inclusive of both males and females but have pooled subjects. In the present day, male-focused research is becoming less acceptable, especially given the substantial number of articles being published across disciplines showing prominent sex and gender differences in the mechanisms contributing to innate behaviors, cognition, and disease. For example, Douton and Carelli (5) evaluated how oscillatory signaling dynamics in rats are involved in negative affect, which is prevalent in various psychiatric disorders, and in addiction. Perhaps one of the most important findings of this article was that while the male and female rats did not differ in innate and learned affective behaviors, oscillatory signaling dynamics within the infralimbic cortex to the nucleus accumbens shell, a pathway known to be involved in negative affect, were dependent on sex. Similarly, using a stress model in rats, Theriault *et al.* (6) showed that the severity and manifestation of depression-like behaviors induced by stress did not differ between males and females (although timing of onset did differ). However, the underlying oscillatory changes that accompanied these established behaviors in each sex were distinct. These preclinical findings are noteworthy as they demonstrate first that distinct oscillatory patterns within each sex may be associated with similar behavioral manifestations, and second that the underlying molecular and cellular mechanisms that drive these oscillatory changes and behaviors are likely distinct in each sex. From a clinical perspective, caution must therefore be taken when considering sex and gender differences in research as the expression of similar behaviors or disorder symptoms may be driven by different mechanisms and may therefore require different pharmacological interventions.

Participant Subgrouping and Data Analysis. As discussed, researchers have long had the tendency to group subjects together, a practice that fails to account for individual variation in biological measures. This lack of information is particularly important when studying oscillatory dynamics due to the known coupling of oscillations with behavior and cognitive processes. In preclinical studies, the subgrouping of behavioral traits can be extremely insightful, giving us important clues on the mechanisms that underlie behavior in both healthy and disordered states. Stress studies are a good

Box 1. Research Considerations in Systems Neurobiology

Sex and Gender
- Incorporate both male and female subjects where appropriate
- Consider sex and gender beyond the binary

Participant Subgrouping and Data Analysis
- Appreciate that many disorders show prominent individual differences in symptom manifestation
- Categorize subgroups based on behavior and symptoms
- Ensure comorbidities are considered
- Extend analysis beyond mean comparisons (e.g., data scatter, regression analyses)
- Incorporate effect sizes
- Consider how data overlap may influence desired outcomes
- Analyze sex and gender as a primary independent variable rather than a covariate

Quality Over Quantity
- Appreciate that the appropriate incorporation of sex/gender and other subgroups will promote scientific advancement
- Reflect on what you are trying to achieve as a researcher and design the experiments accordingly

SEE CORRESPONDING ARTICLE ON PAGE 354

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example of this—in recent years, more studies have analyzed stress-exposed rats using distinct stress-susceptible and stress-resilient groups (6). However, it is not always obvious where subgroups exist or how to consider individual variation. We should therefore reassess how we evaluate our data.

When we perform statistical analyses on oscillatory measures or any other type of data, one of the most common comparisons we make is between group means. However, data are rarely that simple. There is often overlap in the data scatter between groups caused by individual variability. In systems function research, this overlap is a key consideration because it may give insight into the usefulness of certain functional readouts. For example, data scatter in neurophysiological biomarker research may be problematic due to the risk of false positives and/or false negatives. Analyzing effect sizes may be helpful in some instances, especially in situations where the significance value is low and/or within-group variability is high. In addition, regression analyses may be beneficial in elucidating whether there is a relationship between oscillatory activity and behavior. Douton and Carelli (5) showed an overall elevation in mean infralimbic cortex spectral power with aversive behavior in male but not female rats. However, there also appeared to be substantial variability in both the oscillatory and aversive responses. To further elucidate this, they performed a regression analysis between the oscillatory power of each frequency and gaping behavior, where they demonstrated a positive correlation between the power of lower-frequency delta, theta, and beta oscillations in the infralimbic cortex and this aversive behavioral responding. Similar correlation analyses have been performed in stress studies, thus relating depression-like susceptibility and resilience to sex-dependent and region-specific oscillatory dynamics at select frequencies (6). Although correlation does not necessarily mean causation, analyses such as these effectively link oscillatory activity to behavioral output by considering individual subject variability within the dataset.

The same type of subgrouping can, and indeed should, be performed in clinical studies. Major depressive disorder and autism spectrum disorder (ASD) are excellent examples of disorders that manifest as a broad range of behavioral, emotional, and cognitive alterations—yet there is a dearth of clinical research that separates research subjects by these traits. In almost all cases, although demographics are included, research participant symptoms are not described. ASD, for example, is considered a spectrum of disorders, and although many studies that examine circuit function do focus on specific subtypes of ASD, such as fragile X syndrome or Asperger syndrome, most other studies simply group participants together as having autism. Added to this, ASD can be diagnosed over a broad range of ages, with sex-dependent differences, and often males and females of varying ages are also grouped. By separating groups of participants by disorder traits and with consideration of comorbidities, we are much more likely to understand how disorders such as ASD, major depressive disorder, and other disorders manifest.

There are two last key points surrounding data analysis with sex and gender. First, it should be emphasized that sex/gender should not be analyzed as a covariate but rather as an independent variable. Second, if we are to be more inclusive of sex and gender, we should begin to think beyond solely binary measures, understanding that some females will be more "male-like" or vice versa, and give serious thought as to how best to interpret these data with sex/gender as a continuum in mind (7).

Quality is More Important Than Quantity. It may be argued that grouping research participants by behavior or symptoms would add significant time and expense to studies. But have we not historically said the same about sex and gender? After decades of male-focused research, we are only now acknowledging the error of our ways. Unfortunately, we work in a system where productivity is rewarded, such that quantity is often valued over quality. Articles are published with low sample sizes or improper statistical analyses or that offer little in the way of scientific advancement. There are many reasons for this publish or perish mentality, many of which are systemic and are therefore likely beyond the ability of an individual researcher to correct. However, it is sometimes important to step back and reflect on the best ways to find answers to the research questions we seek and whether our research approaches are effective. Douton and Carelli (5) appear to be on the right path when it comes to thinking beyond traditional analytic measures. It is much more likely that we will have scientific breakthroughs if we take our time to ensure that all important factors are considered in our project designs.

A final thought is that in our search for novel oscillatory (and other) biomarkers we should consider the possibility that in some cases a biomarker for an entire neurological disorder may not exist. Rather, disorder biomarkers may be subtype-specific and based on specific measures, such as sex/gender, behavior, symptomology, or the presence of comorbidities. In this regard, as researchers we need to be thoughtful about what we are trying to achieve and be careful in how we design our studies. This is a necessary step in our journey toward personalized medicine.

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