Effects of Stress Across Generations: Why Sex Matters

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In this issue of *Biological Psychiatry*, Saavedra-Rodríguez and Feig (1) demonstrated that the experience of social stress during a period spanning from adolescence to adulthood induces long-term increases in anxiety-like behavior and social deficits in mice. However, the consequences of stress are revealed to extend far beyond this initial effect. Using a breeding design in which stress-exposed males and females are mated with stressed or nonexposed mice (Figure 1), with no subsequent stress exposure, the effects of stress across generations are explored. Offspring (F1), grandoffspring (F2), and great-grandoffspring (F3) were observed to inherit the effects of parental (F0) stress, with females but not males exhibiting anxiety-like behavior and social deficits. Although males do not inherit the behavioral characteristics associated with stress exposure, the breeding design suggested that it is through males (and not females) that these effects can be transmitted to the F3 generation. Both mothers and fathers are capable of the transmission of the effects of social stress to their daughters; however, beyond this F1 generation, it is through stress-exposed fathers that both anxiety and social deficits can be observed in subsequent generations. This study highlights the complex pathways through which males and females may potentially influence the development of future generations and may contribute to a growing literature on the transgenerational consequences of adversity (2).

The notion that the experiences of one generation can have an impact on the development of subsequent generations, leading to vulnerability to psychopathology, has added a new level of complexity to the study of the origins of disease. The question raised by the persistence of environmentally induced effects across generations is regarding mechanism: What biologic and behavioral pathways can lead to the transgenerational continuity of phenotype? It is likely that there is no simple answer to this question, which will require a more in-depth understanding of the interaction between males and females during mating; the prenatal and postnatal interplay between mothers, fathers, and offspring; the molecular events characteristic of the maturation of gametes; and the potential heritability of biological information. Saavedra-Rodríguez and Feig (1) tackled the question of mechanism using a number of approaches. First, they demonstrated that postnatal cross-fostering of F1 offspring between stressed and control parents did not diminish the transmission of the effects of parental stress to offspring. Stress experienced at various times within the life span can result in decreased mother-infant interactions during the postnatal period. Thus, a possible route through which the effects of social stress become perpetuated across generations is through altered maternal care (3). However, it would appear that this behavioral mechanism of transmission does not account for the transgenerational impact of social instability in the current paradigm.

Having eliminated critical factors that can shape postnatal development, including maternal and paternal influences (transmission of behavioral deficits from F1 fathers to F2 daughters is still evident when fathers are absent during postnatal development), what mechanisms that can account for these effects remain? Certainly the prenatal environment plays a significant developmental role and in the case of the maternal transmission of stress effects from the F0 to F1 generation, stress-induced alterations to the hormonal context of fetal development is a candidate mechanism. This pathway is also suggested by the dissipation of the stress-induced effects among the F2 females descendent from the F0 females that experienced stress. However, this leads us to the phenomenon reported by Saavedra-Rodríguez and Feig (1) that is becoming increasingly evident in epidemiologic (4) and laboratory studies (2): the paternal transmission across multiple generations of environmentally induced effects. How is it that mammalian fathers can alter the development of F1, F2, and F3 generations? From the perspective of mechanism, this phenomenon has opened the door to speculation with regard to the inheritance of environmentally induced effects through the germline: a pathway that conjures Lamarckian theories and a degree of skepticism in a DNA-centric era.

Since the 1890s, August Weismann's germ plasm theory of reproduction has become dogma within theories of inheritance with its associated inference that although somatic cells can undergo change in response to the environment, the same is not true of germ cells. Since Weismann's theoretical contributions to the study of inheritance, the discovery of 1) DNA structure, 2) the mechanics of DNA replication, and 3) the process of meiosis has shifted the focus to genetics as the sole biologic mechanism of inheritance and led to the dismissal of the inheritance of acquired characteristics. However, as Saavedra-Rodríguez and Feig (1) demonstrated, the inheritance of acquired characteristics, in this case characteristics that may lead to vulnerability to psychiatric dysfunction, is alive and well but likely mediated by mechanisms that were not easily predicted by the observations of Weismann and Mendel. There is increasing evidence for the heritability of epigenetic modifications (5): molecular changes that alter the expression of genes without altering the underlying DNA sequence. Because these mechanisms also exhibit plasticity in response to a broad range of environmental exposures (i.e., nutrition, stress, maternal behavior, toxins), epigenetic pathways provide a promising target to explore within the context of transgenerational effects. Although the current study does not explore these pathways, previous studies have demonstrated environmentally induced epigenetic variation in male germ cells with similar modifications present in the brain and sperm of F1 offspring that, in some cases, are observed in the F2 and F3 generations (2,6). These heritable epigenetic changes may account for the increased *Rcan1* and *Rcan2* expression levels in stress-exposed mice and their F1, F2, and F3 daughters reported by Saavedra-Rodríguez and Feig (1). Disentangling those molecular and gene expression changes that are a consequence of inherited epigenetic variation from those that mediate the inheritance itself will be a significant challenge for future studies of this phenomenon.

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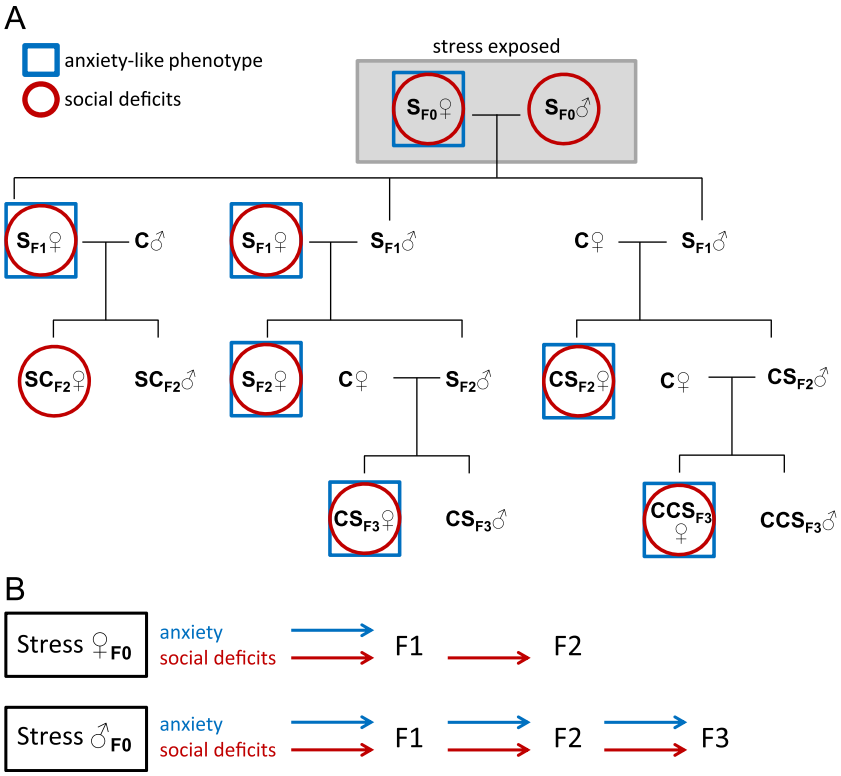

Figure 1. Summary of research design and transgenerational effects reported by Saavedra-Rodríguez and Feig (1). **(A)** In this study, stressed (S) males and females (S_{F0}♂, S_{F0}♀) were mated to generate stressed F1 offspring. F1 offspring were then either mated with control (C; nonstressed) mice or F1 offspring of stressed mice to generate the F2 generation. F2 mice were then mated with either the F2 descendants of stressed mice or with controls to generate the F3 generation. Blue square indicates an observed increase in anxiety-like behavior (relative to control mice), whereas a red circle indicates deficits in social behavior. **(B)** Summary of the transmission of the effects of social stress to F1, F2, and F3 generation females (male phenotype was not altered). Through the matriline, increased anxiety-like behavior is observed through to the F1 generation (social deficits persist until the F2 generation). Conversely, in the patriline, anxiety-like behavior and social deficits persist to the F3 generation. S, stressed; S_{F0}♂, stressed males; S_{F0}♀, stressed females; C, control; F1, offspring; F2, grandoffspring; F3, great-grandoffspring.

Perhaps one of the most perplexing issues raised by Saavedra-Rodríguez and Feig (1) is the sex specificity of the effects of social stress. In all generations, it is females and not males that are susceptible to social stress (experienced or historic), whereas it is males that are capable of transmitting stress susceptibility across multiple generations. This finding would suggest that although stress induces changes in somatic cells in females, leading to behavioral and neurobiologic changes, among males, stress targets the germ cells rather than the somatic cells leading the transmission of behavioral and neurobiologic changes to future generations. What can account for this sex-specific division of labor? Sex differences in susceptibility to environmental adversity is well documented (7) and likely the norm rather than the exception. Moreover, there may be many mechanistic pathways to account for these sex differences, including epigenetic variation. However, in the case of the inheritance pattern suggested by the current study (Figure 1), why would the male germline be modified by social stress and the female germline be protected from this effect? Ultimately, this may be an issue of timing and the difference in the time course and plasticity of molecular events occurring during gamete maturation. The timing of de novo DNA methylation (an epigenetic modification critical for growth and development) within germ cells may occur at different time points within the life history of males versus females and within different cellular phases dependent on the sex of the individual (8). The de novo DNA methylation is thought to be a key epigenetic process through which the biologic embedding of experiences occurs, and thus, sex differences in epigenetic events in the germ cells may account for the different patterns of inheritance observed in matrilines and patrilines (2,4,6). If we also consider the phenomenon of imprinted genes, genes that are expressed in a parent-of-origin manner such that offspring express only the maternal or paternal copy of a gene (9), then we are left with a cascade of molecular events in which the transmission of adverse environmental exposures depends on both the sex of the parent that experienced the event and the sex of the individual that is receiving this ancestral biologic information. Regardless of how this is achieved, it is clear that sex matters, and through careful consideration of how males and females are differentially affected by the environment and the diverse pathways through which males and females can influence development across generations, we will attain a deeper understanding of the origins of disease risk.

Where do we go from here? In the quest to understand the impact of adversity within and across generations, there are fundamental questions regarding mechanism that need to be addressed. With rapid advances in the study of epigenetics and increased availability of methodologic approaches to study of these mechanisms, it may be possible to further explore these pathways in transgenerational research designs. However, equally important will be careful consideration of the prenatal and postnatal factors and maternal-paternal interactions that may mediate or moderate the transmission of traits across generations. Inheritance of behavioral variation and disease risk induced by environmental stressors is likely to involve multiple pathways, and by approaching the question of mechanism from a perspective that is inclusive of these different modes of heredity, it may be possible to generate more accurate predictions regarding the origins of phenotype (10).

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