Biological Psychiatry: Celebrating 50 Years

Commentary

Extinction Versus Epigenetic Intergenerational Inheritance: Who Wins?

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The effect of environmental stressors on parents and the subsequent influence on the offspring of these parents is a topic of wide interest to both the scientific community and the wider population in general. It is a topic that is likely to cause its own stress in the contemplation of how our experiences will negatively impact our children and perhaps even our grandchildren. Although we cannot control our genetics and the transmission of our DNA, might we be able to influence our epigenome and thus save our progeny from undesirable consequences of what we have experienced? In the current issue of *Biological Psychiatry*, Aoued *et al.* (1) not only describe the transmission of a behavioral sensitivity to progeny but also show, for the first time, that a behavioral intervention in a parent can block the intergenerational inheritance of this behavioral sensitivity. Overall, the intriguing results open a new avenue of study on reversing intergenerational inheritance of maladaptive behaviors.

Intergenerational inheritance refers to the transmission of a phenotype acquired in the parental generation (F0) that is induced by an environmental stimulus or particular experience and that is then passed along to the next generation (F1) in males (or from the F0 to the F2 in females, because stimuli in utero can affect both the F1 offspring and its gametes, which would then cause changes in the F2 offspring). It is often referred to as a parental effect and should not be confused with transgenerational inheritance, which refers to the transmission of a phenotype from one generation to at least two generations beyond (e.g., the F2 generation of a male or the F3 generation of a female) without presentation of the stimulus in the intervening generation (2). There is a growing body of literature documenting the intergenerational and transgenerational reprogramming of bodily systems, including metabolic processes, allergic diseases, and, more recently, brain development (3–5). Although the evidence of environmental and experiential effects on offspring is growing, a need to find interventional strategies in parents to reverse these nongenetically inherited changes opens a new therapeutic avenue.

Dias and Ressler (6) previously demonstrated that mice can be trained to associate mild foot shocks with an odorant and that sensitivity to the associated odorant could be transferred to both the F1 and F2 generation in male mice. In the current study, Aoued *et al.* (1) focus on the intergenerational inheritance (F0 to F1) of sensitivity to odorants in which parents learned to associate the two different odorants (acetophenone and Lyral) with mild foot shock. In the F1 progeny, sensitivity to an odorant was measured using an acoustic startle task. Thus, if a parent mouse formed an association between an odorant and the shock, the F1 progeny would exhibit an enhanced startle response to an acoustic stimulus in the presence of that odorant (even though they had never experienced that odorant).

Indeed, that is what the authors observed: F1 offspring exhibit behavioral sensitivity to an odorant that they had never experienced. To understand why, the authors examined the expression of the receptor for the odorant and found that F1 offspring have increased levels of the receptor and that the gene encoding the receptor had decreased levels of DNA methylation (in the sperm of the F0 offspring). A decreased level of DNA methylation in a gene promoter region is usually associated with increased expression of that gene. The most exciting aspect of the study by Aoued *et al.* comes from the interventional strategy to reverse the behavioral sensitivity to this odorant in F1 progeny.

After being trained to associate the odor with mild foot shock, a third group of animals (F0-extinguished) was put through an extinction protocol wherein the animals ceased to freeze in response to odor presentation (similar to the F0-exposed only group) (Figure 1). When the F1 generation of the F0-extinguished group was tested for an odor-potentiated startle response, they no longer showed a behavioral sensitivity, and were similar to the F1 of the F0-exposed group. This is the first demonstration of the ability to use extinction-based approaches to reverse the effect of environmental stressors on parents transmitted to their offspring.

The molecular mechanism for intergenerational transfer of this type of behavioral sensitivity is hypothesized to be epigenetic. As genetic material is not altered, some sort of transcriptional reprogramming may be occurring, and that information is subject to epigenetic regulation, such as DNA methylation, histone modification, and changes in noncoding RNA, such as microRNA, small interfering RNA, and Piwi-interacting RNA (7). Defining a specific epigenetic mechanism for the transfer of information remains problematic because all of these regulators undergo some sort of reprogramming during development of the gametes, development of the early embryo, or both. To address the issue of mechanism, Aoued *et al.* (1) looked at DNA methylation at the promoters of the genes encoding the receptors for the odorants (acetophenone and Lyral) in the F0 sperm.

DNA methylation is an epigenetic mechanism whereby a methyl group is added to DNA, which can modify the expression of nearby genes. In this study, Aoued *et al.* (1) examined the methylation of cysteine (5-methylcysteine), which is a commonly studied DNA modification. Aoued *et al.* (1) were able to detect a decrease in methylation of the odorant receptor promoters in the sperm of the F0-trained group, and

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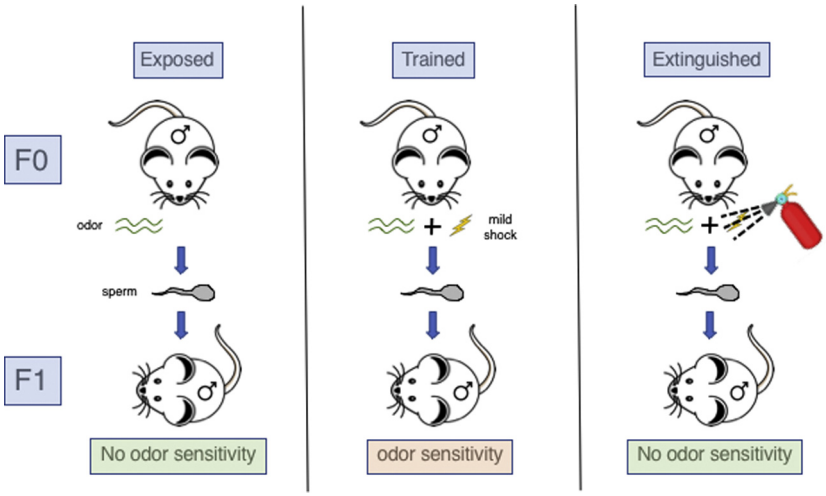

Figure 1. Extinction training ameliorates odor sensitivity in F1 offspring. In the F0-trained group, animals learned to associate an odor with mild foot shock, which led F1-trained offspring male mice to develop an odor sensitivity, as measured by an odor-potentiated startle response. The control F0-exposed group only received exposure to the odor, and F1-exposed male offspring exhibited no odor sensitivity. Similarly, animals that received extinction training in the F0-extinguished group sired male F1-extinguished offspring that exhibited no odor sensitivity.

levels of methylation returned to that of the F0-exposed group in the F0-extinguished group. These results suggest that extinction can reverse the hypomethylation status at the promoters of these genes. Indeed, protein levels of the receptors, with hypomethylated promoter regions, were increased as measured by Western blot analysis as well as examination of the size of the glomeruli for the odorant receptor in F1-trained mice, and this phenotype was reversed in the F1-extinguished mice.

Overall, the results of this study show not only that a stress-induced associative learning event can be transmitted to the F1 generation (in the form of a behavioral sensitivity to an odor) but also that a signature of changes to protein expression and neuroanatomy (glomeruli changes) can be observed that define the behavioral expression of the transmitted odor information. Furthermore, the association between the odor and stress (mild foot shocks) can be extinguished by behavioral interventions in the F0 generation, which subsequently protects the F1 generation from inheriting a behavioral sensitivity to the odorant—this is an impressive finding.

It is important to note, as acknowledged by Aoued *et al.* (1), that DNA methylation has its own problems as a mechanistic determinant of intergenerational and transgenerational inheritance because the genome undergoes two rounds of demethylation during development: once during gametogenesis and once early in embryogenesis. The process of demethylation is proposed to be a mechanism of establishing totipotency of the embryo to enable full body patterning without the influences of parental experiences. By its very nature, it is a mechanism to prevent the transfer of environmental influences onto progeny. In fact, for the paternal genetic material during embryogenesis, the demethylation process is faster and more complete than it is for the maternal genetic material, making a purely methylation-centric epigenetic mechanism seem unlikely (8).

Regardless, the odor/stress paradigm used in this study sets a strong foundation to dissect the molecular mechanism of intergenerational epigenetic inheritance as well as extinction approaches to reverse transmitted information. One of the most pressing questions is whether similar phenomena will be observed in females. In the current study, inheritance was assayed exclusively in male progeny, but understanding whether the behavioral sensitivity can be transferred to females is imperative to understanding the molecular mechanisms of this type of associative learning transmission. Dias and Ressler (6) previously showed that F0 females trained to associate an odorant with foot shock could also transmit this sensitivity to F1 male progeny, but F1 female progeny were not examined. This raises interesting questions with regard to the mechanism by which odor sensitivity is being transmitted and whether it is different from F0 males to F1 males as compared with F0 females to F1 males.

Regarding the current study, the mechanism involves transmission via sperm, yet there remain key open questions. Sperm take up to 2 months to fully mature in the mouse, but they are constantly renewed throughout the life of the animal. Thus, it would be important to know how long the ability to transmit the odor sensitivity phenotype lasts. Does the associative learning event in F0 fathers affect only the sperm in the process of maturation at the time of the learning event, or are all sperm capable of transmission for the life of the animal?

A final thought on this topic is whether an experience must be stressful to engage epigenetic machinery for transmission. Is the transmitted information and resulting behavioral sensitivity always going to be a positive adaptive response, or might it also lead to negative and perhaps inappropriate responses? This field of research is new, and the questions are boundless, but the investigation of intergenerational and transgenerational epigenetic inheritance will undoubtedly prove interesting, startling, and extremely important to our understanding of cognitive function, behavior, and influence on brain disorders as well.

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Article Information

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