# Mechanisms of Transgenerational 'Memory' in Mice and Rats: A Review

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**Abstract:**

Researchers have discovered that parental experiences and environment can affect offspring behaviors and phenotypes through non-genetic mechanisms in one or more generations among various animals. This phenomenon is most commonly studied in mice and rats, where different mechanisms are believed to exist due to the diverse inheritance patterns discovered with different parental experiences in the experiments, including stress, liver damage, odor fear conditioning, environmental enrichment (EE), etc. Besides the commonly known factor, social transmission, epigenetic modifications are also suggested to be a major cause, with DNA methylation, histone modification, and microRNA level change observed in different studies. The epigenetic marks are lost after certain generations, and they are found to be reversible due to environmental change. This non-genetic phenotypic response to environmental stimuli could benefit or harm offspring.

**Keywords:** stress, environmental enrichment, methylation, histone, microRNA

## 1. Introduction

Transgenerational 'memory' is the behavioral or phenotypic changes in response to an environmental stimulus that could be passed down from parents to their offspring using non-genetic mechanisms. The mechanisms should affect offspring survival rates in a fast-changing environment, either positively or negatively [1], and they provide evidence for the longly dismissed Lamarckism [2]. This non-genetic way of transgenerational information transmission has been found to exist in various animals, including Caenorhabditis elegans (C. elegans) [3], flies [4], mice, and rats [5-7]. Notably, each type of animal is shown to employ a unique mechanism, and various responses are elicited differentially, even within the same animal species. Due to the numerous distinct research focuses in this field, the correlations between different research findings are often neglected, which could be the key to understanding a shared transgenerational 'memory' strategy. Furthermore, although experiments have been sophisticatedly designed to investigate the non-genetically inherited phenotypes, many changes related to behavioral patterns are quite hard to quantify [5]. Consequently, the accuracy of observations, theories, and the exclusivity of the theories, etc., are all possible obstacles in this field.

In recent years, environmental inheritance has also been considered an important component of psychiatric disease pathogenesis, as suggested by the findings from mouse and rat experiments. Parental behaviors could affect subsequent generations positively or negatively, depending on the persistence of environmental changes [1]. Interestingly, this non-genetic inheritance can occur before conception or without any social contact with the affected parents [6, 8]. Thus, in this article, I will focus on the research on mice and rats, as the mechanisms of different species differ greatly and as the mouse experiments may have greater medical importance to humans. By reviewing the transgenerational 'memory' phenomena in these small mammals, similarities and controversies in different research with different parental stimuli should be revealed. New hypotheses and future directions should be highlighted.

## 2. Responses to Aversive Stimuli

Most research on mice and rats has focused on how traumatic experiences affect subsequent generations [6, 9]. At the same time, only a few looked at environmentally enriched mice, where the parents experienced cognitive training and physical exercises [5]. The mainstream hypothesis for the transgenerational 'memory' mechanism is the change in epigenetic marks, and evidence in DNA methylation, microRNA level change, and histone mark modifications have been discovered in different types of transgenerational 'memories' [5-7].

## 3. Stress

Parental exposure to early life stress was found to affect the coping behaviors in the subsequent generations [1]. A report by Gapp et al. [10] used the stress-addition method called MSUS, where they made females isolate and remove newborn pups (F1) unpredictably from the dams with additional stress on the dams during separation.

Then, the light-dark box and fixed ratio paradigm were performed on the MSUS male offsprings and their male offsprings to observe the behavioral changes. As predicted, the mice with parental stress resisted aversive conditions and had a more active coping response.

Moving on to the changes in gene expression, as indicated by previous studies, the glucocorticoid receptor (GR) facilitated the rapid termination of the stress response, and methylation was identified as a regulatory mechanism for the GR gene [11]. For this knowledge, Gapp et al. [10] performed quantitative RT-PCR (qRT-PCR) to measure GR expression, and they also performed labeling and mass spectroscopy to examine proteins on a proteome-wide scale in the MSUS F1 and F2 generations. Compared with the control, a GR overexpression was discovered, which results in functional changes in the hippocampus, indicated by the alterations of glucocorticoid response-related proteins. Then, the researchers used quantitative bisulfite pyrosequencing (qBSP) to detect methylation, where they found DNA hypomethylation at the hippocampus GR gene of F1 and F2 and in F1 sperm cells. Interestingly, when Gapp et al. [10] went further to investigate whether this DNA methylation was maintained in different tissues of the offspring, the qBSP in the offspring prefrontal cortex (PFC) showed different results from those in the hippocampus, indicating that DNA methylation changes in sperms were not consistently passed on to all cells in the offspring.

Gapp et al. [10] proved that DNA hypomethylation could affect offspring behaviors, at least in the case of parental exposure to early life stress, which supported the epigenetic hypothesis further. The finding that DNA methylation differed in different regions and tissues also posed the new question of what mechanism is in effect when the epigenetic marks are transferred transgenerationally [10]. This is especially intriguing as epigenetic marks are known to be erased globally during spermatogenesis and embryonic development [12, 13]. Meanwhile, it is also worth mentioning that the F1 pups were returned to the dams, which meant that there might be a social transmission component in the causes for their behavioral change. Plus, the research focused on male offspring, while transgenerational 'memory' was also observed when the mothers were affected [8]. It thus left the question of whether the mechanisms of paternal and maternal transgenerational 'memories' were the same due to the differences between oocytes and sperms. For example, somatic components within the oocyte and the in-utero environment may affect epigenetic changes in offspring [7, 14], the size differences of cytosol between the germ cells may affect the amount of epigenetic-modifying molecules carried, and the time of generation of oocytes and sperms are different, etc.

## 4. Liver Damage

Zeybel et al. [7] designed an experiment by repeatedly administering hepatotoxin carbon tetrachloride ($CCl_4$) to induce liver fibrosis through hepatic healing to investigate the epigenetic hypothesis of liver cirrhosis pathogenesis. Using olive oil as a control for $CCl_4$, four groups of F2 rats were obtained. Group A's ancestors were controlled using olive oil; group B's fathers were injured, but grandfathers were controlled; group C's fathers were controlled, but grandfathers were injured; group D's ancestors were all injured using $CCl_4$. All four groups of F2 were then injured. While the ancestral liver injury did not affect offspring $CCl_4$ metabolism or its induction of liver fibrosis, an intergenerational and a transgenerational suppression of wound healing response in the liver were observed. They also tested whether the repression works for other tissues using renal injury. They showed that this mechanism does not affect fibrogenesis globally in an offspring mouse, suggesting not confirming a tissue-specific change.

The cellular basis of healing suppression was the decrease in a specific type of myofibroblasts, which arose from activating hepatic stellate cells. Zeybel et al. [7] research showed on the molecular level, compared with the control, there was a higher expression in PPAR-γ and α, which were repressed during stellate cell activation [15]. Meanwhile, a decreased expression of TGF-β1 induced stellate cell activation [16] in group D rats, whose ancestors were injured. Besides, several genes involved in fibrogenesis regulation and the transdifferentiation of hepatic stellate cells also showed significant overexpression or underexpression in group D [7, 17, 18].

Zeybel et al. [7] also found epigenetic evidence in the rat livers. By pyrosequencing, less methylation of the PPAR-γ gene was found at CpG2, CpG3, and CpG4, with group D having the lowest methylation, whereas for TGF-β1 gene, a slightly higher level of methylation at four CpG sites was discovered in group D than group A. Plus, the results from the quantitative chromatin immunoprecipitation (qChIP) analysis revealed that there were elevated levels of acetylated H3 at the gene promoter of PPAR-γ in group D than in group A, and the acetylation of H3 at the gene promoter of TGF-β1 was reduced in group D.

Moving on to sperms, rats with liver fibrosis exhibited an increased level of the histone variant H2A.Z, which was known to be correlated with reduced levels of DNA methylation at specific genomic regions, suggesting that H2A.Z incorporation into nucleosomes might act as a suppressor of CpG methylation [12, 13, 19]. The

trimethylation of histone H3 at Lys27 (H3K27me3) also increased within the chromatin of the PPAR-γ gene. Furthermore, they also conducted a serum transfer experiment from the injured to uninjured rats. Fascinatingly, this experiment showed a small increase in PPAR-γ–associated H3K27me3 and a notable increase of H2A in the naive serum recipient, indicating that the modifications in sperm chromatin are transferable.

Zeybel et al.'s [7] results provided evidence of DNA methylation and histone modification for transgenerational memory. They suggested a possible transport pathway of the molecule that can modify chromatin structure or epigenetic marks - the serum. They also further highlighted the relationship between DNA methylation and histone mark modifications, which affect each other's presence [19]. The inhibitory effect of a histone variant presence on DNA methylation may serve as the mechanism to reverse an offspring phenotypic change, as it may not always be beneficial [1]. It should also be mentioned while Gapp et al. [10] showed a non-global change of DNA methylation in offspring, Zeybel et al. revealed a non-global fibrosis in offspring. The similarity between the findings might suggest a similar epigenetic mechanism. Zeybel et al. [7] also mentioned that the somatic information from mothers may affect epigenetics in offspring, which was the reason why they chose males; however, similar to Gapp et al. [10], the maternal influence needs to be investigated later so that we can know whether the findings are a collaborative effect of paternal and maternal influences.

## 5. Odor Fear Conditioning

The specificity of the olfactory system makes odor a useful tool in studies. In the olfactory system, one odorant receptor (OR) typically correlates with one gene, and multiple olfactory sensory neurons (OSNs) with the same OR converge to the same glomerulus [20], which allows relatively direct observation of the effect of a specific odor on the olfactory system. With this background, Dias and Ressler [6] conducted an odor fear conditioning experiment where F0 mice received an electric shock when a specific odor was present, and the responses of their subsequently conceived offspring to the ancestral conditioned odor were tested. Two different odors, acetophenone, and propanol, were used in their experiment, with the prior knowledge that M71 odorant receptors correlating to the Olfr151 gene were activated by acetophenone [21]. When F0 mice were treated with acetophenone, their F1 and F2 expressed increased sensitivity and fear only with acetophenone but not propanol, and vice versa. Besides, an IVF experiment and a cross-fostering experiment were also done to eliminate the social transmission of the fear response, indicating a non-genetic inheritance via parental gametes.

The neuroanatomical cause for this, according to Dias and Ressler [6], was that M71-specific glomeruli in the OB of offspring with acetophenone-treated ancestors significantly increased in size compared with the control, accompanied by a significant escalation in the amount of M71 OSNs. On the molecular level, they found that the Olfr151 gene displayed reduced CpG methylation in the sperm DNA of both F0-conditioned males and F1 naive mice, as observed through bisulfite sequencing. They also performed native chromatin immunoprecipitation (N-ChIP). Still, no differences in histone-mediated epigenetic signatures were found, possibly due to an inappropriate antibody choice in N-ChIP or a non-histone-based mechanism.

The findings of Dias and Ressler [6] showed the effect of DNA methylation at a specific locus on offspring phenotypic and behavioral changes in response to an odor, and the special thing about their experiment was that they successfully eliminated the effect of social transmission. Some of the questions left by the research, including the transport pathway of epigenetic-modifying molecules, histone modifications, etc., may be suggested by the findings of other researchers [7]. Szyf [2] also pointed out in his review of Dias and Ressler [6] that there should be currently unidentified sensors within gametes that can detect and incorporate brain signals into specific regions of the sperm genome to link an odor with an inheritable fearful experience. Specifically, the GR and microRNA mentioned by Szyf [2] have been proven to relate to transgenerational 'memory' with different environmental stimuli [5, 10].

## 6. Response to Environmental Enrichment (EE)

Environmental enrichment (EE) combines physical and cognitive training [5]. Arai et al. [8] discovered that EE of juvenile female mice enhanced long-term potentiation (LTP) in their offspring using a signaling cascade involving cAMP/p38 MAP kinase, which is known for introducing a supplementary signaling input during the initiation of LTP [22]. Unlike other transgenerational 'memories,' this augmentation is intergenerational, meaning it only lasted for one generation after F0. Besides, they also discovered that mouse memory impairments associated with a specific mutation could be masked or improved in their offspring when the mutated parents are exposed to EE, which might indicate a medical potential in humans.

Arai et al. [8] limited their results to juvenile animals.

Benito et al. [5] then experimented on adult mice where an intergenerational pattern of LTP, synaptic plasticity, and cognition enhancement was observed, but a different molecular explanation was given. Benito et al. [5] found microRNAs (miRs) 212/132 increases in the hippocampus and sperms of F0 EE mice, but no increase was found in their offspring (F1). To confirm the effect of miR212/132, they also conducted an experiment using three groups of mice-sperm-RNA-injected oocytes: EE mouse sperm RNA with vehicle (scrambled RNA) injected oocyte, home cage (HC) mouse sperm RNA with vehicle-injected oocyte, and EE mouse sperm RNA with miR212/132 inhibitors injected oocyte. Only the mice developed from the first group showed a significant enhancement, meaning that miR212/132 are the main causes for this non-genetic inheritance.

Another influence of EE was discovered by Gapp et al. [10] when they researched transgenerational 'memory' of early life stress. They found that EE MSUS F1 mice responded similarly to EE control F1 mice, but EE MSUS F2 mice showed a more spontaneous escape behavior. On the molecular level, the increase in hippocampal GR mRNA expression and the hypomethylation of the GR gene in the hippocampus and sperms were well reversed by EE, indicating that paternal EE effectively prevents the transgenerational transmission of behavioral symptoms.

The EE research has shown several mechanisms of non-genetic transgenerational inheritance [5, 8, 10]. Although the findings of Arai et al. [8] seem to be by Benito et al. [5], the cAMP/p38 MAP kinase is known to not be induced by EE in adults [22], which leaves the question unanswered on the similarity between the two mechanisms. Similar to Benito et al.'s [5] research, studies of other stimuli may suggest candidates for the pathway of chromatin-modifying molecules and histone modifications of EE mice [7]. In addition, the reversal effect of EE on gene expression is also not fully understood. Still, it is a plausible mechanism since studies have suggested that transgenerational 'memory' can sometimes be harmful [1]. Generally, studies on transgenerational 'memory' follow the research steps from offspring phenotypic changes to the protein level (gene expressions) and then epigenetics (possible explanations for gene expression change). Among the findings, DNA hypomethylation seems to be the most common epigenetic reason, followed by DNA hypermethylation, histone acetylation, and microRNA level changes. The involvement of signaling pathways, including the kinase signaling pathway [10], and an unknown odor-fear combining circuit that might involve GR, sperm olfactory receptors, and microRNAs [2] were also mentioned. Furthermore, it was also suggested that different mechanisms might depend on each other, as DNA methylation-histone modification mutual exclusivity has been discovered [19].

## 7. Conclusion

Various non-genetic inheritance mechanisms have been discovered in mice and rats with different ancestral stimuli, including changes in DNA methylation and histone H3 acetylation, induction of kinase signaling cascade, and increase in microRNA levels, which affect offspring survival rates and signify the alternative mechanisms of inheritance that are not based on the gene sequence alone. Questions regarding the accuracy of experiment conclusions, the applicability of the discovered mechanism, and whether the mechanisms are separate are left to be answered. Meanwhile, new side discoveries accompanied by the studies, including the mutual exclusivities of histone modifications and DNA methylations, are also captivating.

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