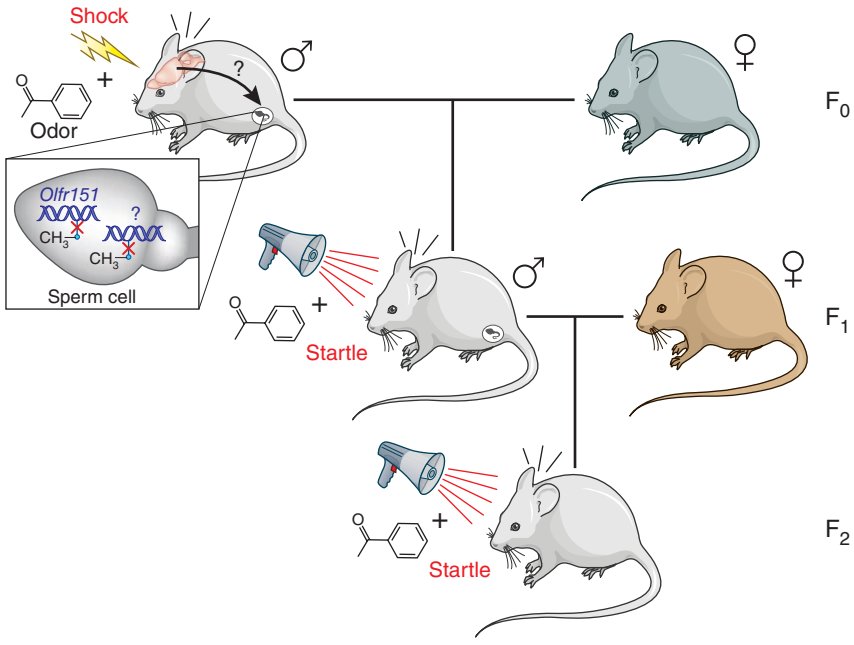
Lamarck revisited: epigenetic inheritance of ancestral odor fear conditioning

Moshe Szyf

A study shows that when mice are taught to fear an odor, both their offspring and the next generation are born fearing it. The gene for an olfactory receptor activated by the odor is specifically demethylated in the germ line and the olfactory circuits for detecting the odor are enhanced.

Odor sensitivity has been a critical driving force in evolution. Specific recognition of distinct odors is important for the survival of the species and enables individuals to escape predators, identify nutritional resources and gain protection from hazards, as well as form social bonds and choose mates. A good example of odor sensitivity that is critical for survival through avoidance of predators is the rodent fear response to fox urine[1]. Predator odor selectivity is thought to be generated during evolution through natural selection of sequence variations in the olfactory receptors encoding new odor selectivities. A report by Dias and Ressler[2] in this issue of *Nature Neuroscience* offers an additional, non-genetic mechanism for this quintessentially evolutionary affair.

The olfactory system is uniquely positioned to address the question of nongenetic inheritance. Each olfactory receptor neuron, originating in the nasal epithelium, expresses only a single member of the olfactory receptor gene family, and the olfactory neurons that respond to a particular odor can be mapped and identified[3]. Dias and Ressler[2] modeled an ecologically relevant exposure by pairing an odor with mild foot shocks. They trained mice to fear the odor acetophenone, which is recognized by Olfr151, and then measured the behavioral response to this odor in future generations (**Fig. 1**). As a control, they used a different odor that was not paired with shocks, propanol, which acts on a different receptor, Olfr6. Notably, the authors found that, when mice were trained with acetophenone, the F_1 and F_2 generations showed a heightened startle response in the presence of acetophenone, but not in the presence of propanol. When the ancestors were instead trained with propanol, their descendants were fearful in the presence of propanol, but not acetophenone. The authors showed that the response was transmitted through either the male or female germ line up to two generations, suggesting that sperm and egg DNA register the exposure as an epigenetic mark.



Katie Vicari

Figure 1 Model for epigenetic inheritance of odor fear conditioning. Association of acetophenone odor with an electrical shock conditions the mouse for an enhanced acetophenone startle response. Although the mechanism is unknown, this may trigger the release of circulating molecule(s), such as microRNAs or glucocorticoids, that act on spermatogonia to direct DNA methylation changes in both specific olfactory receptor genes, such as *Olfr151*, and other genes, as yet unknown, that are involved in the fear conditioning circuitry in the brain. When the demethylated sperm fertilizes a naive female, the methylation pattern is maintained in the fertilized eggs and may guide the differentiation of fear circuitry. The adult F_1 mouse exhibits enhanced startle in the presence of acetophenone. During primordial germ cell differentiation in the F_1 mouse, the methylation pattern triggered by the conditioned exposure to acetophenone is preserved. When the resulting marked sperm fertilizes a naive mouse, the offspring F_2 will develop the same conditioned fear response circuitry in the brain, using the epigenetic information in the F_1 sperm to guide differentiation. The adult F_2 mouse likewise shows a heightened startle response in the presence of acetophenone.

Moshe Szyf is in the Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada.
e-mail: moshe.szyf@mcgill.ca

Because a molecular target, a specific olfactory receptor gene, is known, the authors were able to examine epigenetic marks at this locus. They found that there were differentially demethylated sites in *Olfr151*, but not in *Olfr6,* when the mice were trained to fear acetophenone. This exquisite targeting of DNA methylation in sperm in response to a specific exposure is unprecedented and startling. Even more surprising is the fact that this methylation signature in sperm is transferred to the F_1 and F_2 generations, indicating that it escapes both the post-fertilization and primordial germ cell erasures of DNA methylation. Such specificity of germline transmission of an adaptive behavior in response to a distinct signal has been generally believed to come into existence only through natural selection. These data suggest that epigenetic, transgenerational, germline-transmitted adaptations to threat and changes to phenotype occur in a predictable and organized fashion, similar to that of other physiological responses. The authors provide strong evidence that these changes are indeed transmitted through the germ line and not behaviorally. The strongest line of evidence comes from an *in vitro* fertilization experiment, with sperm from a specific odor–conditioned mouse resulting in transmission of an anatomical marker, the increased size of odor-specific glomeruli in the olfactory bulb.

Researchers have long asked whether there are mechanisms that can translate adult experience and environmental exposures into inherited phenotypes without affecting the genotype. A related question is whether these mechanisms subserve long-term 'directed' adaption in which one generation signals to the next generation how to adjust genetic programs to cope with the distinct environmental and experiential challenges that are anticipated. As environments are dynamic, it is clear that genomes must adapt to these changes in some manner. The main evolutionary mechanism of adaptation is through natural selection, whereby variants in the sequence of DNA that confer a fitness advantage in coping with new challenges are selected for, leaving others by the wayside. The idea that random genetic mutations are kept or discarded through natural selection has been dominant in our biological and social thinking for over a century. Linked with this concept is the idea of genetic determinism: that our phenotype is predetermined by our inherited and naturally selected genome. The possibility that environmental exposures could guide or direct transgenerational phenotypic adaptations in the absence of genetic selection, as implied by Lamarckism, has traditionally been dismissed. The main reason for rejection of this hypothesis has been the lack of clear examples of pure Lamarckism and a mechanism that could serve as a conduit between the environment and stable alteration of gene function that could be stably transmitted through the germ line.

Epigenetic mechanisms stably program gene function in the absence of sequence changes[4]. These mechanisms include modification of the DNA molecule itself by methylation[4] and hydroxymethylation[5], as well as chromatin modification[6]. Experimental evidence that has accumulated in the past decade suggests that epigenetic mechanism can alter gene function in response to the environment in the brain and somatic tissues[7]. If the same mechanisms are in effect in the germ line, then epigenetic changes in sperm or egg might occur in response to environmental exposures and then transmit the experience of that exposure to future generations through the germ line. However, this hypothesis faces two challenges from entrenched concepts in the field. The first idea is that any epigenetic marking of the germ line would be lost in the F_1 generation immediately after fertilization[8]. The second notion is that any remaining marking would be lost during the differentiation of primordial germ cells[9], eliminating any possibility of the transfer of epigenetic information to the F_2 generation.

However, in spite of this common wisdom, several epidemiological studies in humans and a growing number of studies in animal models suggest a nongenetic germline transmission of exposure to pesticides and experience, including metabolic deprivation, increased fat intake, depression, cocaine addiction or fear across generations[10]. A few of these studies found evidence that the germline transmission went a generation beyond possible direct exposure of the germ line. However, the remaining question is whether these are specific organized responses that direct distinct phenotypic consequences or whether the parental exposure increases stochastic noise in the epigenome that is then transmitted across generations, resulting in an altered distribution of phenotypes.

The tantalizing data presented by Dias and Ressler[2] leave us with critical and perplexing issues. First, the authors did not find a DNA methylation mark in the particular olfactory receptor genes in olfactory response neurons, where one would anticipate that the epigenetic memory would express itself. Second, epigenetic marks are inherently tissue specific. There is a long series of differentiation steps between sperm and olfactory receptor neurons, which must involve many stages at which cell type–specific DNA methylation patterns are sculpted. How is the DNA methylation mark in the sperm maintained through these steps to guide the expression of the phenotype in the appropriate neurons and not in other cells? This is even more perplexing in that it seems that this DNA methylation mark is absent in the olfactory receptor neurons themselves. Third, not just the memory for the odor, but also the association of the odor with a fearful experience, is transmitted across generations. This should involve a more complex neuronal circuitry than just the olfactory receptor. What are the epigenetic marks in the sperm that are transmitted to the particular neurons involved in this linking of fearful response to an ancestral odor exposure? Fourth, how does the germ cell recognize and integrate both the olfactory signal and fearful experience? Is there a channel of communication between the CNS and specific loci in the gametes?

The authors propose that germ cells, which are known to contain olfactory receptors, are activated by odor and trigger a signaling pathway that targets site-specific DNA methylation. However, this is not sufficient to explain how these changes in DNA methylation in olfactory receptors are linked to the fearful experience. Thus, there must be sensors of behavioral experience in the gamete that are as yet unknown and that could incorporate the brain signals into several particular addresses in the sperm genome (**Fig. 1**). Sperm olfactory receptors could be a component of such machinery, as well as hormone receptors such as glucocorticoid receptors. Other attractive candidates include microRNAs, which could potentially circulate systemically from brain to sperm and target specific sequences in the genome. A microRNA was recently proposed to mediate transgenerational response to paternal stress[11]. The changes in DNA methylation must be protected in the germ line and transmitted during cellular differentiation to guide the formation of particular circuitry and anatomical densities of olfactory receptors during brain development. The lack of differential methylation in the mature olfactory receptor neuron might indicate that these differentially methylated gene targets are critical for the developmental stages and disappear once the relevant circuitry is established (**Fig. 1**).

Unraveling the molecular link between experience and the gamete epigenome, and the relationship between gametes and the development of behavioral brain circuitry in response to experience, is bound to be a formidable challenge. Nevertheless, Dias and Ressler's data[2] force us to rethink our understanding of phenotypic adaptation, as well as entrenched ideas on how species respond to

new challenges. Their results suggest that evolution has equipped organisms with mechanisms to respond specifically and efficiently to certain critical novel experiences, such as odor and predator threat, and to transmit this information effectively to their offspring without the need for the typically slow process of natural selection. It is unclear how prevalent this mechanism is and whether it evolved only to respond to ecological challenges such as odor. An interesting question is whether there is a mechanism that could then fix these epigenetically driven phenotypic changes in the genetic sequence, thereby altering the course of evolution. The study by Dias and Ressler[2] provides strong evidence that the germline can serve as a vector for transmitting lessons from adult experience across generations. Future studies are needed to determine how important these mechanisms are in humans and whether they influence the rapid evolution of phenotypes seen in human populations.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

1. Anisman, H., Hayley, S., Kelly, O., Borowski, T. & Merali, Z. *Behav. Neurosci.* **115**, 443–454 (2001).
2. Dias, B.G & Ressler, K.J. *Nat. Neurosci.* **17**, 89–96 (2014).
3. Buck, L. & Axel, R. *Cell* **65**, 175–187 (1991).
4. Razin, A. & Riggs, A.D. *Science* **210**, 604–610 (1980).
5. Ficz, G. *et al. Nature* **473**, 398–402 (2011).
6. Turner, B.M. *Cell* **111**, 285–291 (2002).
7. Szyf, M. *Clin. Genet.* **81**, 341–349 (2012).
8. Reik, W., Dean, W. & Walter, J. *Science* **293**, 1089–1093 (2001).
9. Hajkova, P. *et al. Mech. Dev.* **117**, 15–23 (2002).
10. Grossniklaus, U., Kelly, B., Ferguson-Smith, A.C., Pembrey, M. & Lindquist, S. *Nat. Rev. Genet.* **14**, 228–235 (2013).
11. Rodgers, A.B., Morgan, C.P., Bronson, S.L., Revello, S. & Bale, T.L. *J. Neurosci.* **33**, 9003–9012 (2013).

How sex prevents violence: the magic of caress (and GABA)

Liming Wang

Long-term exposure to females reduces aggression of male fruit flies. The mechanism involves contact-dependent pheromone sensing and the activation of a small group of GABAergic inhibitory neurons unique to the male brain.

Although conspecific aggression is an integral part of animals' social lives, uncontrolled aggression is usually harmful[1]. Previous social experience imposes a robust regulatory effect on aggression[2,3], which may help to suppress excessive aggression in a socially enriched environment and maintain a stable social structure. The mechanisms underlying the inhibitory effect of social experience on aggression remain largely unclear, especially at the level of central neural circuitry. In this issue of *Nature Neuroscience*, Yuan *et al.*[4] tackle this question by examining whether and how prior social experience with females influences male-male aggression in the fruit fly *Drosophila melanogaster*. Yuan *et al.*[4] found that housing with females for an extended period of time exerted an inhibitory effect on male aggression toward other males. The inhibition likely involves contact-dependent pheromone sensing and the subsequent activation of a small group of GABAergic inhibitory neurons unique to the male brain. These results offer an entry point to understanding how social experience shapes aggressive behavior in the CNS (**Fig. 1**).

The presence of female flies is known to impose an acute stimulatory effect on male-male aggression in fruit flies[5], highlighting the possible role of male aggression in establishing advantage in mate competition[6]. But does prolonged prior exposure to females exert a similar or opposite effect on subsequent male aggression? Yuan *et al.*[4] found that, if male flies were kept with females for 24 h before the aggression assay, these males no longer exhibited enhanced aggression toward each other following acute female exposure. These results indicate an inhibitory effect of chronic female exposure on male aggression in fruit flies. It would be of interest to further explore whether this inhibitory effect occurs by counteracting the stimulatory effect of acute female exposure on male aggression or by reducing baseline male aggression.

The inhibitory effect of chronic female exposure on male aggression could be a result of behavioral interactions between males and females (for example, courtship and copulation) or of long-term exposure to female-specific sensory cues. Yuan *et al.*[4] therefore sought to distinguish these two possibilities. First, Yuan *et al.* found that copulation was neither necessary nor sufficient for the inhibitory effect of chronic female exposure on male aggression. Second, chronic exposure to *D. melanogaster* and *D. pseudoobscura* females, but not to *D. virilis* females, suppressed male aggression, even though *D. melanogaster* males avidly courted the females of all three species. Thus, prolonged behavioral interactions with females are unlikely to be the sole cause of reduced male aggression. Notably, *D. pseudoobscura* and *D. virilis* females carry distinct profiles of long-chain cuticular hydrocarbons, a class of non-volatile pheromones involved in the regulation of both sex and aggressive behaviors in fruit flies[7,8]. In addition, Yuan *et al.*[4] found that physical contact between males and females was essential for the inhibitory effect of chronic female exposure on male aggression, whereas visual and olfactory input were dispensable. Taken together, these results suggest that contact-dependent communication via cuticular hydrocarbons may mediate the inhibitory effect of chronic female exposure on male aggression.

What specific cuticular hydrocarbon molecule mediates the suppression of aggression by chronic female contact? Plausible candidates emerged from Yuan *et al.*'s results[4]. They found that ppk29 (ref. 9), a contact-dependent gustatory receptor, was required for the inhibitory effect of chronic female contact, as were gustatory neurons that coexpressed ppk29 and fruitless (fru), a master regulator of fly sex and aggressive behaviors. Previous reports have shown that these ppk29$^+$ fru$^+$ double-labeled neurons are located on the bristles of fly forelegs and are sexually dimorphic[9]. These ppk29$^+$ fru$^+$ neurons respond to a wide array of cuticular hydrocarbons from both males and females, including 7-tricosene, 7-pentacosene, 7,11-heptacosadiene (7,11-HD), 7,11-nonacosadiene (7,11-ND) and possibly others[9,11]. One or more of these cuticular hydrocarbons may therefore mediate the suppression of male aggression by chronic female contact by activating ppk29$^+$ fru$^+$ neurons. Two of these compounds, 7,11-HD and 7,11-ND, are female specific and have been shown to promote male courtship[9]. It is therefore possible that the same female pheromones (for example, 7,11-HD and 7,11-ND) may promote courtship and suppress aggression via the same gustatory pathway.

How does the female pheromonal signal enter the male brain and suppress aggression?

Liming Wang is at the Life Sciences Institute, Zhejiang University, Hangzhou, China.
e-mail: lmwang83@gmail.com