# The Inherited Scent of Fear

*The Dias–Ressler Olfactory Conditioning Experiment, Its Evidence for Intergenerational Transmission in Mice, and the Debate That Followed*

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## Abstract

In a 2014 paper in *Nature Neuroscience*, Brian G. Dias and Kerry J. Ressler of Emory University reported that male mice trained to fear the odor acetophenone (a sweet, almond- or cherry-blossom-like scent) sired offspring, and grand-offspring, that were unusually sensitive to that same odor although they had never encountered it. The descendants also had more olfactory neurons tuned to acetophenone, and the fathers' sperm carried reduced DNA methylation at the gene for the acetophenone receptor. In-vitro fertilization and cross-fostering controls argued that the effect travelled through the germ cells rather than through social learning. This paper describes the experiment in technical and plain terms, evaluates its strengths and weaknesses, reviews the statistical critique by Gregory Francis and the mechanistic objections of epigeneticists such as Edith Heard and Robert Martienssen, and surveys subsequent work: the Ressler–Dias laboratory's 2019 demonstration that extinguishing the parent's fear prevents transmission, the independent Columbia University study (Liff et al., eLife) that corroborated the neuroanatomical effect, sperm-RNA studies by Isabelle Mansuy and others, and the much weaker human evidence. The conclusion is that the core neuroanatomical finding has gained partial independent support, the behavioral and molecular claims remain less secure, and the mechanism by which an odor experience could reach sperm is still unknown.


## 1. Introduction

Since August Weismann proposed in the 1880s that the germ line (sperm and egg) is sealed off from the body's experiences, biology has largely rejected the Lamarckian idea that acquired traits can be inherited. Genes, not life

experiences, are passed on. Yet clinicians who treat trauma have long observed that the children of traumatized parents show elevated rates of anxiety and stress disorders. Most of that pattern can be explained by shared genes, upbringing, and family environment. The open question is whether any part of it is transmitted biologically through the germ cells themselves.

Dias and Ressler designed their experiment to isolate exactly that possibility. Their key insight was to use the sense of smell, because the mouse olfactory system offers something rare in behavioral biology: a direct, mapped line from a single chemical to a single receptor gene to an identifiable set of neurons in the brain. That precision allowed them to ask not merely whether "stress" is inherited, but whether information about one specific cue in a parent's environment can show up in the offspring.

## 2. Background

### *2.1 How a mouse smells: the one-receptor, one-neuron rule*

The mouse genome contains roughly 1,100–1,400 functional odorant-receptor genes. Each olfactory sensory neuron (OSN) in the nasal lining, the main olfactory epithelium (MOE), expresses just one of these genes, chosen largely at random during the neuron's development. All neurons expressing the same receptor send their axons to the same one or two spots in the olfactory bulb of the brain, called glomeruli. Acetophenone strongly activates the receptor M71, encoded by the gene Olfr151. In genetically engineered M71-LacZ mice, every M71 neuron and its glomerulus can be stained blue, so researchers can count the neurons and measure the glomeruli directly.

> **In plain language**
> Think of the nose as a switchboard with about a thousand kinds of telephone line, each answering one family of smells. Acetophenone rings one particular line, M71. In the special mice used here, every M71 line can be dyed blue, so scientists can literally count how many M71 lines a mouse has and how big their junction box in the brain is.

### *2.2 Epigenetics, and the difference between inter- and transgenerational effects*

Epigenetic marks are chemical tags that change how genes are read without changing the DNA sequence. The best studied is DNA methylation: methyl

groups attached to cytosine bases at “CpG” sites, which generally silence nearby genes. Less methylation (hypomethylation) usually means a gene is more available for use. Other candidates include histone modifications and small non-coding RNAs carried in sperm.

Terminology matters for evaluating the claim. When a male is exposed, the sperm that will form his offspring (F1) are directly exposed, so an F1 effect is called *intergenerational*. The F2 generation (grandchildren) is the first that was never directly exposed in any form, so a paternal-line effect in F2 qualifies as *transgenerational*. (For a pregnant female, the threshold is F3, because the fetus’s own germ cells are exposed in the womb.) The Dias–Ressler F2 data are therefore the most important part of their claim.

### *2.3 Why most biologists expected a negative result*

Mammals wipe most DNA methylation twice: once in the early embryo shortly after fertilization, and again in the developing germ cells of the embryo. Heard and Martienssen (Cell, 2014) note that these two rounds of erasure leave little opportunity for environmentally induced marks to survive, and that proof of a genuinely epigenetic basis for transgenerational inheritance in mammals is generally lacking. Any positive result must therefore explain how a mark escapes both waves of reprogramming.

## 3. The Experiment

### *3.1 Design*

Adult male mice (the F0 generation), both ordinary C57BL/6J mice and M71-LacZ reporter mice, were trained by classical (Pavlovian) fear conditioning: an odor was repeatedly paired with a mild foot shock over several days, so that the odor alone came to predict the shock. One group was conditioned to acetophenone, which activates M71; a comparison group was conditioned to propanol, which does not activate M71; a further group stayed in the home cage. The conditioned males were then mated with untrained females. Their offspring (F1) and grand-offspring (F2) were raised without ever being exposed to either odor.

### *3.2 How “fear” was measured*

The behavioral test was odor-potentiated startle. A mouse is startled by a sudden loud noise, and the size of its startle jump is recorded. If a smell makes the animal anxious, the startle is larger when that smell is present. The score compares the startle with the odor against the startle without it. Because this measures

heightened reactivity, it does not by itself distinguish inherited fear from inherited sensitivity to the odor, a limitation the authors acknowledged.

### *3.3 Controls for non-genetic routes*

- **Odor specificity.** Offspring of acetophenone-trained fathers were tested with both odors, as were offspring of propanol-trained fathers. A general increase in anxiety would show up for both odors; a specific inherited effect would show up only for the father's odor.
- **Paternal transmission.** Male mice contribute nothing to rearing, so passing an effect through fathers removes most maternal-care explanations.
- **In-vitro fertilization (IVF).** Sperm from conditioned males was used to fertilize eggs in a dish, ruling out any influence of the trained father's behavior, smell, or interaction with the mother during mating.
- **Cross-fostering.** Offspring of conditioned mothers were raised by unconditioned foster mothers, to rule out learning from the mother after birth.
- **Second generation.** F2 mice were bred from F1 males that had never smelled acetophenone.
- **Blinding.** In their later reply to critics, the authors stated that experiments were run blind and analyzed in a double-blind manner.

## 4. Results

The findings were consistent across levels of analysis:

- **Behavior.** Offspring of acetophenone-trained fathers startled more strongly in the presence of acetophenone but not propanol; offspring of propanol-trained fathers showed the mirror-image pattern. In one representative M71-LacZ experiment, groups of 9–13 male offspring were compared, with the acetophenone effect reaching significance at $P = 0.003$ by analysis of variance.
- **Neuroanatomy.** F1 offspring of acetophenone-trained males had larger M71 glomeruli in the olfactory bulb and more M71-expressing neurons in the nasal epithelium than controls. F2 grand-offspring also had enlarged M71 glomeruli.
- **IVF.** Offspring conceived by IVF with sperm from acetophenone-trained males had larger M71 glomeruli than offspring of propanol-trained males.

- **Cross-fostering.** Offspring of conditioned mothers remained sensitive to the conditioned odor and had enlarged M71 glomeruli even when raised by untrained foster mothers.
- **DNA methylation.** Bisulfite sequencing found reduced CpG methylation at Olfr151 in the sperm of acetophenone-trained fathers (compared with propanol-trained fathers) and in the sperm of their F1 sons. However, no such hypomethylation was found in the olfactory neurons of F1 or F2 mice themselves.

| Generation | What happened to it | Reported outcome |
|---|---|---|
| F0 (fathers) | Trained: acetophenone + mild shock | Fear of the odor; more M71 neurons; Olfr151 hypomethylated in sperm |
| F1 (children) | Never exposed to the odor | Greater startle to acetophenone only; larger M71 glomeruli; more M71 neurons; Olfr151 hypomethylated in sperm |
| F1 via IVF | Conceived in a dish from F0 sperm | Larger M71 glomeruli |
| F2 (grandchildren) | Bred from naive F1 males | Greater behavioral sensitivity to acetophenone; larger M71 glomeruli |

> **In plain language**
>
> The fathers learned that a cherry-blossom smell meant a shock. Their pups, and then their grand-pups, had never smelled it, yet they jumped harder at a loud noise when the smell was in the air, and their noses had extra "cherry-blossom" detector cells. This happened even when the pups were made by test-tube fertilization or raised by a different mother, so the pups did not simply learn it from their parents. The fathers' sperm showed a chemical change on the very gene that detects that smell.
>
> Note what was not shown: the grandchildren did not "remember" the shock. What passed down looks more like a tuned-up sensitivity to one smell than a story-like memory.

# 5. Critiques

## *5.1 "Too much success": Gregory Francis*

Gregory Francis, a Purdue University psychologist who specializes in detecting publication bias, published a critique in *Genetics* (2014). His argument is statistical. Experiments with modest sample sizes and modest effects should fail to reach significance some of the time even when the effect is real. Using the effect sizes the paper itself reported, he estimated that the probability of all ten behavioral experiments succeeding was only about 0.023. The implication is that unreported null experiments or flexible analysis may have inflated the results.

Dias and Ressler replied in the same issue. They stated that every experiment conducted was reported, no data were excluded, testing was blind, and peer review had added further experiments, both positive and negative. The exchange did not settle the question; Francis's method flags a pattern as improbable but cannot prove misconduct or error, and critics of the method note that it rarely clears a paper.

### 5.2 The mechanism problem

The deeper objection is biological. For the result to be epigenetic, at least three things must happen, none yet demonstrated:

- A signal from the nose and brain must reach the testes and alter one specific gene (Olfr151) in developing sperm, and not other genes.
- That mark must survive the two waves of epigenetic reprogramming described in Section 2.3.
- In the offspring, the mark must bias which receptor developing olfactory neurons choose, even though the hypomethylation was not detected in those neurons.

Heard and Martienssen also list alternative explanations that studies in this field often fail to exclude: undetected DNA mutations, behavioral transmission, microbiome differences, and transmission of metabolites. The IVF and cross-fostering controls in Dias and Ressler address the behavioral and microbial routes reasonably well, but not every route.

### 5.3 Other limitations

- Sample sizes per group were small (roughly 9–13 animals in key comparisons), which magnifies the statistical concern.
- The methylation differences were measured at a small number of CpG sites and were not shown to cause the anatomical change.
- The behavioral test measures sensitivity or reactivity; it does not establish a fear memory as such.

- Most early follow-up came from the same laboratory, which is not independent replication.

# 6. Follow-up Studies

## *6.1 Reversing the effect: Aoued, Sannigrahi, … Ressler and Dias (2019)*

In *Biological Psychiatry* (2019), the same research group, now with Dias running his own lab at Emory's Yerkes center, trained fathers to fear either acetophenone or a second odor, Lyral (which activates the receptor MOR23). One group then underwent extinction training, repeated exposure to the odor without shocks, until the fear faded. Offspring of the extinguished fathers showed no behavioral sensitivity and no enlarged M71 or MOR23 representation, unlike offspring of fathers who were trained but not extinguished. The authors reported that extinction also removed the sperm signature. The study was explicitly designed in part to replicate the 2014 F1 results, which it did, though it is not independent.

If valid, this is striking: the parent's later experience reversed the mark before conception. The researchers highlighted the possible relevance to treating parents with post-traumatic stress before they have children.

## *6.2 Independent corroboration: Liff et al., Columbia University (eLife)*

A team from Bianca Jones Marlin's laboratory at Columbia University (first author Clara W. Liff) repeated olfactory fear conditioning with acetophenone and Lyral using double-reporter mice. They confirmed that conditioning increases the number of neurons tuned to the conditioned odor in the parents and found that this increase was detectable in F1 offspring. Using a cell-birth label (EdU), they argued the mechanism in the parent is a bias in which receptor newly born olfactory neurons choose, not merely better survival of existing cells. The behavioral differences they found in offspring were more nuanced than in the original paper, and the authors discuss the discrepancies with Dias and Ressler. eLife's editorial assessment rated the evidence as solid and concluded that the effect is partially transmitted across generations.

This is the most important development since 2014: an independent laboratory corroborated the core neuroanatomical finding in F1. It did not test F2, and it did not establish the behavioral result in the original form.

## *6.3 Sperm RNA: an alternative carrier*

Several groups now favor small non-coding RNAs in sperm, rather than DNA methylation, as the carrier. Gapp, Mansuy and colleagues (*Nature Neuroscience*, 2014) subjected male mouse pups to unpredictable maternal separation and stress. Injecting RNA purified from these males' sperm into normal fertilized eggs reproduced the behavioral and metabolic changes in the resulting offspring, a strong causal test. Rodgers, Bale and colleagues (2013) reported that paternal stress alters sperm microRNAs and reprograms offspring stress hormones. Work from the Rando and Chen/Zhou groups (both in *Science*, 2016) showed that small RNAs can be loaded into sperm as they mature in the epididymis, offering a possible route from the body to the germ line that bypasses DNA reprogramming. Whether such a route explains an odor-specific effect is untested.

### *6.4 A clearer case in worms*

The best-established case of inherited learned behavior is in the nematode *C. elegans*. Moore, Kaletsky and Coleen Murphy (Cell, 2019) showed that worms that learn to avoid a pathogenic bacterium pass that avoidance to their progeny for about four generations, mediated by small RNAs. Worms, however, lack the extensive germline reprogramming of mammals, so the result shows the phenomenon is biologically possible without showing it in mammals.

## 7. What About Humans?

Popular accounts often connect the mouse study to human trauma. The human evidence is much weaker, for an unavoidable reason: researchers cannot separate biological transmission from shared genes, family environment, and parenting in human families.

- **Dutch Hunger Winter.** Heijmans and colleagues (PNAS, 2008) found that people conceived during the 1944–45 Dutch famine had altered methylation at the IGF2 gene six decades later. This is an in-utero (prenatal) exposure, not germline inheritance.
- **Holocaust survivors.** Rachel Yehuda and colleagues (Biological Psychiatry, 2016) measured methylation of the stress gene FKBP5 in 32 survivors and 22 adult offspring. Survivors showed higher methylation at one site and offspring showed lower methylation at the same site. Critics pointed to the very small control group (8 parents and 9 offspring) and to the opposite direction of change, which is not what simple inheritance of a mark would predict.

Bernhard Horsthemke (Nature Communications, 2018) concluded in a critical review that there is no convincing evidence yet for transgenerational epigenetic inheritance in humans. The cautious reading is that intergenerational effects of parental stress are plausible and partly documented, while true transgenerational epigenetic inheritance in humans remains unproven.

## 8. Assessment

| Claim | Strength now | Basis |
|---|---|---|
| Parental odor conditioning increases odor-specific neurons in F1 | Moderate | Original study; same-lab replication (2019); independent corroboration (Liff et al.) |
| Effect passes via gametes, not social learning | Moderate | IVF and cross-fostering in original study; not independently repeated |
| Odor-specific behavioral sensitivity in F1 | Weak to moderate | Original and same-lab data; independent behavioral results more nuanced; statistical critique |
| Effect persists to F2 (transgenerational) | Weak | Original study only |
| Olfr151 sperm hypomethylation is the mechanism | Weak | Correlation at few CpG sites; absent in offspring neurons; no causal test |
| Comparable inheritance in humans | Unproven | Small, confounded studies; reviews by Horsthemke and others |

The experiment is best understood as an elegant, carefully controlled, but small study that produced a surprising result. Twelve years on, its central anatomical observation has gained some independent support, which is more than many sensational findings receive. The behavioral claim and especially the F2 “grandchildren” claim still rest essentially on the original data, and the proposed molecular mechanism is unconfirmed. The honest scientific position is open but cautious: something about a father’s sensory experience appears to reach his offspring in mice, and nobody yet knows how.

## 9. Conclusion

Dias and Ressler did not overturn Weismann, but they reopened a door that most biologists considered closed. Their use of a single odor mapped to a single receptor gave the field a rare, testable model of inheritance of a specific acquired

trait. The best summary of the state of the evidence is that the effect is real enough to have been partly reproduced, controversial enough that its statistics and mechanism are still disputed, and far from established in humans. The most important future work would be an independent, preregistered, adequately powered replication that includes the F2 generation and a direct causal test of the carrier, whether DNA methylation or sperm RNA.

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