The expression of parental fear-conditioned memory in the hippocampal neurons of subsequent progeny

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Abstract.

This paper researches the concept of parental fear-conditioned memory and its potential transmission to subsequent generations, focusing on examining whether memory traces formed in the hippocampus CA3 region of parents are retained in the hippocampus of their offspring. Through experiments conducted on mice, it is expected that the findings will reveal how fear conditioning in the parental generation impacts the behavioral sensitivity and neuroanatomical changes in the hippocampal neurons of the offspring. The study explores the potential inheritance of transgenerational memory from parents to children and the specific brain sites involved.

Keywords: Parental fear-conditioned memory, transgenerational inheritance, epigenetic modifications

Introduction

Responding to environmental stimuli is a survival instinct for organisms to survive normally in their environment. This is usually expressed as changes in the nervous system. On the one hand, this survival instinct may not be stored and transmitted as a memory, or it may be stored as a memory in the hippocampus CA3 [1].

Some formative memories of the parents are also reflected in the offspring, such as the sense of smell and form synapses in the brain. Memory formation is associated with the reactivation of CA3. DNA methylation in mouse promoter-associated CpG islands (CGI) can be transmitted from parents to offspring [2]. These phenomena suggest genetic mechanisms beyond gene-environment interactions, such as epigenetic modifications, DNA methylation alterations, histone and chromatin modifications, and small regulatory RNA alterations. [3] Despite the overwhelming evidence of direct environmental effects on epigenetic modifications, whether environmentally induced epigenetic changes are transmitted from generation to generation in humans and the form in which they are transmitted remains a matter of debate [3].

Although the mechanism of transgenerational inheritance has not been fully elucidated, it is biologically plausible. Most maternal DNA methylation is reprogrammed during early embryogenesis and germ cell development. However, some DNA methylation signals, such as imprinted genes, may evade this process [3]. Other epigenetic mechanisms, such as histone and ribosome modifications, have the potential to be transmitted directly through the mother. Studies on transgenerational epigenetic inheritance for other nerves in humans are still sparse [3] even though it has been extensively researched in animal models [4].

This essay investigates the expression of parental fear-conditioned memory in the hippocampal neurons of subsequent progeny.

Parental trans-generational memory

Fear conditioning is commonly used in laboratory animals to investigate fear responses and determine whether parental transgenerational memory is inherited. Similar methods are used to investigate this phenomenon [5].

The study discovered that olfactory fear conditioning in the F0 generation had subsequent effects on the adult F1 and F2 generations [1].

For the behavioral sensitivity test, both groups of mice, F0-Ace and F0-Prop, received shocks during conditioning with acetophenone or propanol. [1] Then, homozygous M71-LacZ transgenic male mice or sexually inexperienced and odor-naive C57Bl/6J male mice were employed. The mice were either left in their home cage or subjected to propanol or acetophenone conditioning. Subsequently, conceived adult male offspring (F1) were divided into three groups: F1-Home, F1-Ace, and F1-Prop. [1] None of the F0 males were excluded from the study after training; all were mated with naive females. Mice carrying the M71 odorant receptor in their olfactory epithelium and able to detect acetophenone include the C57Bl/6J and M71-LacZ strains. The olfactory sensory neurons (OSNs) of the M71-LacZ mice produce β-galactosidase in M71-expressing neurons, allowing for visualization.

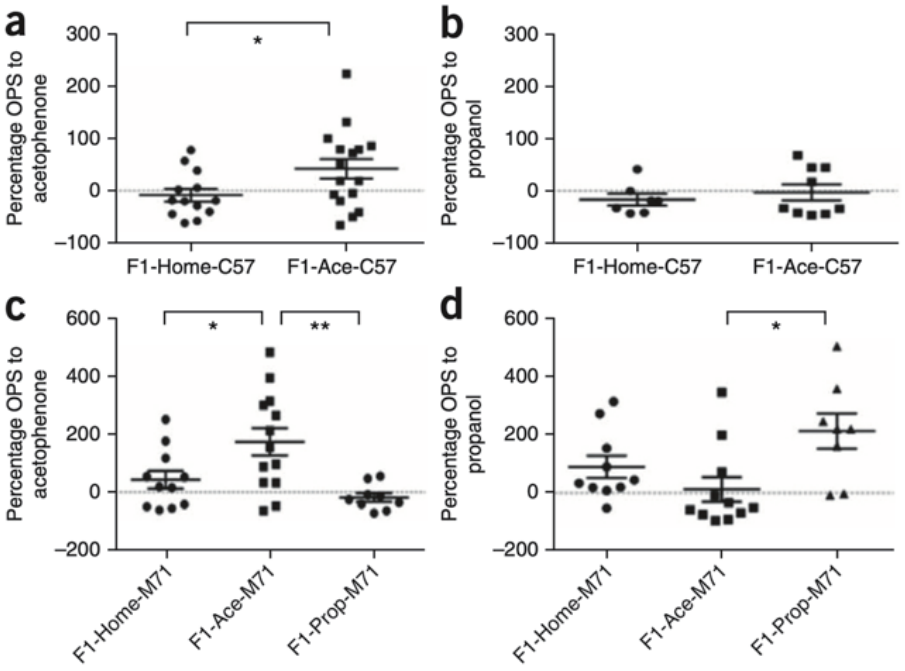

Figure 1. When an odor is combined with a startle stimulus, an enhanced OPS score is calculated, reflecting a larger startle to the odor compared to the control [6].

The F1 offspring from the F1-Ace-C57 showed a heightened sensitivity to acetophenone, but not to propanol, compared to the F1 offspring from F1-Home-C57. Similarly, in another experiment with M71-LacZ F1 males, the F1 offspring from acetophenone-conditioned fathers (F1-Ace-M71) exhibited an enhanced sensitivity to acetophenone but not to propanol. In contrast, the F1 offspring from F1-Prop-M71 displayed an increased sensitivity to propanol but not to acetophenone. In the test of neuroanatomical changes, F0 mice were subjected to odor fear conditioning before conception, as shown in Figure 1.

The F1 and F2 generations were then assessed for behavioral sensitivity to the odor that was conditioned in the F0 generation. Standard β-galactosidase staining was employed to examine any alterations in the neuroanatomical representation of the conditioned odor in naive M71-LacZ F1 males. Specifically, the dorsal and medial M71-specific glomeruli in the olfactory bulb were measured in the F1 offspring of acetophenone-trained F0 males and compared to those of offspring from F0 males trained either in the home cage or with propanol. Additionally, the numbers of M71 olfactory sensory neurons (OSNs) in the main olfactory epithelium (MOE) were quantified.

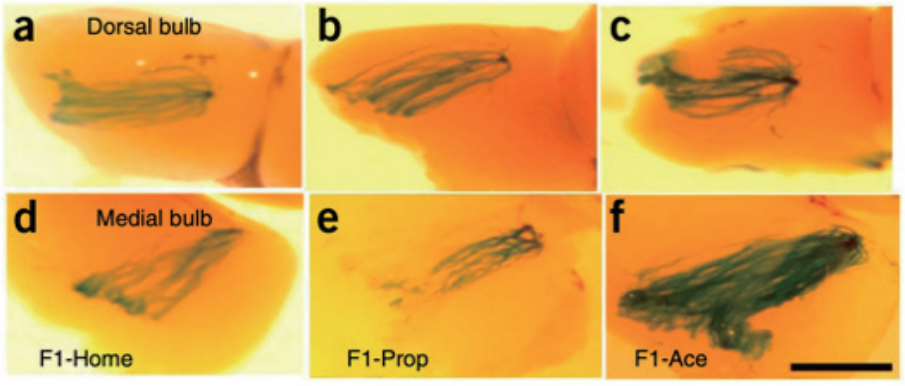

Figure 2. Representative images of the dorsal olfactory bulb of F1 generation mice with different stimuli [6].

Quantitative studies indicate that the F1 generation's behavior was more sensitive to the F0-conditioned

odor than it was to other odors. To be more precise, an improved neuroanatomical depiction of the Olfr151 pathway was used to supplement the F1 generations' behavioral sensitivity to acetophenone. As demonstrated in Figure 2, the olfactory bulb of the F1 children of acetophenone-trained F0 males had considerably bigger dorsal and medial M71-specific glomeruli than did the olfactory bulbs of the offspring of home cage- or propanol-trained F0 males.

Trans-generational epigenetic modification

Transgenerational epigenetic modifications are essential for information to be inherited across generations, impacting gene expression and phenotypic outcomes. However, unlike genetic mutations, which involve changes in the DNA sequence, epigenetic modifications involve chemical modifications to DNA or histone proteins that can silence genes. These modifications are expected to have effects on offspring.

DNA modification

The covalent addition of methyl groups to DNA nucleotides is a well-known epigenetic mark. [Maximilian H. Fitz-James1 and Giacomo Cavalli1 †] Firstly, methyl can be added to DNA, thereby preventing transcription proteins from attaching to DNA. In addition, the CG, CH, and CHH motifs on DNA can also be methylated, where H corresponds to nucleotides other than G. The methylation of DNA can also be caused by adding a methyl group to the DNA. [7] Although methylation also occurs in invertebrates and fungal species, it is in vertebrates and plants that DNA methylation plays the most prominent role in transcriptional regulation and is, therefore, most likely to result in transgenerational genetic phenotypes [2].

Histone modification

In eukaryotic DNA packaging, linear DNA tightly wraps around histones, requiring enzymes to temporarily separate DNA from them to allow transcription. The most common form of histone modification is histone acetylation. When an acetyl group is added to histone proteins, it results in tighter packing around DNA, thereby turning the gene off. In eukaryotic DNA packaging, linear DNA is tightly wound around histones, and enzymes are required to temporarily separate the DNA from the histones for transcription. The most common form of histone modification is histone acetylation. When acetyl groups are added to histones, they cause tighter packing around the DNA, which shuts down genes. Many histone modifications have features that lend themselves to TEI in addition to the well-known duty of regulating DNA expression outside of the well-known role of gene expression. These include the potential for self-propagation and diffusion of epigenetic signals through the coupling of "read" and "write" functions [2].

For example, two modifications frequently implicated in trans-generational epigenetic inheritance are the inhibition of the marks histone H3 lysine nine trimethylation (H3K9me3) and H3K27me3, which in many cases are inextricably linked to other epigenetic signals, and, in vertebrates, H3K9me3 is closely associated with DNA methylation [8].

Non-coding RNA

Transgenerational epigenetic modifications encompass diverse regulatory mechanisms, including the involvement of small non-coding RNAs involved in gene regulation both transcriptionally and pre-transcriptionally. [Maximilian Fitz-James, Giacomo Cavalli]. At the transcriptional level, several types of RNAs can silence genes by different mechanisms, namely the small interfering RNA (siRNA)42 and PIWI-interacting RNA (piRNA) [3,9,10].

Neurobiological Mechanisms of trans-generational memory

One way to influence the growth of the adult nervous system is that the parent's exposure to external influences during conception is an effective way for the parent to tell the offspring to respond to future external stimuli. This memory is partly present in the parent's hippocampus. We used ArcCreERT2 bacterial artificial chromosome (BAC) transgenic mice for our experiments to test our hypothesis by simultaneously stimulating the parents. We can see the neurons in the hippocampus that are stimulated to respond to the fluorescence staining and judge whether the parent's memory is passed to the offspring by the fluorescence color development.

Based on the above data, we wanted to use the fear stimulation test, the contextual fear conditioning (CFC), to investigate whether the memory traces formed in the hippocampus CA3 after parental stimulation are still reflected in the hippocampus of the offspring. We want to use this epigenetic change to investigate whether transgenerational memory can be passed from parents to children and study the specific site of action in the brain.

The expression of transgenerational memory in hippocampus CA3 neurons of children whose parents were exposed to a CFC is expected to increase compared to children whose parents were not exposed to CFC [1,6].

Methodology

Standard housing and dark housing. Mice were kept in the light-dark colony cage, which has an average temperature of about 20 degrees Celsius, for 12 hours, and some food and water were provided. [Denny, C. A.] For the behavioral test, the mice were placed in a dark condition for three days and then got back to the ordinary room.

For the memory expression experiment, it was important to ensure that the cages were always the same for every individual mouse. For all tests, the mice were isolated.

To allow the electric shock to mice to successfully lead to fear, freezing behavior is used as an indicator of its level [6].

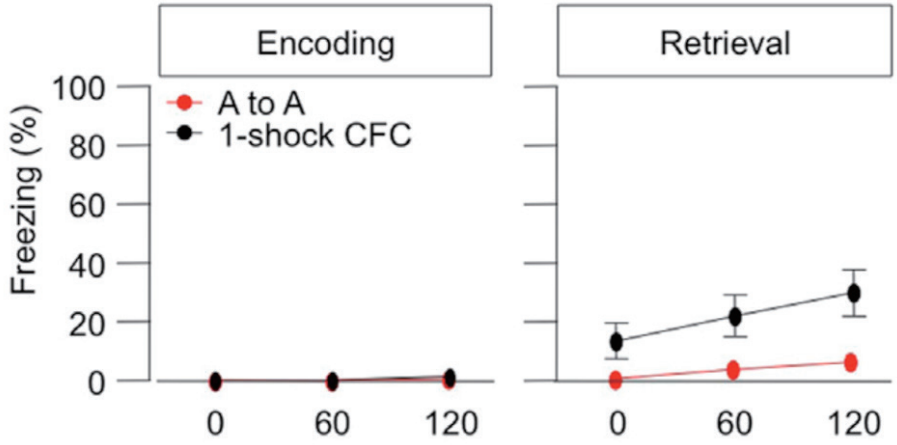

Figure 3. These figures show mice without context have much lower freezing levels than those administered a one-shock CFC. [6]

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One-shock CFC—Duration: 180 seconds, then were removed 15 seconds after the shock. Three shock CFCs—180,240 and 300 seconds were removed 15 seconds after the 300 seconds' shock.

Both one-shock and three-shock CFC were based on research conducted by Wiltgen et al. [6,11].

Dominant Interaction

The indicated dominating interaction was carried out [5]. However, mice were given 30 minutes to explore before the first 10 minutes were recorded and analyzed. Sessions were filmed and examined using behavioral monitoring software (TopScan, CleverSys). This program recognizes a mouse's form, including its nose, body, and tail, to accurately score the number and length of contact with either an item or a mouse. [6]

Immunohistochemistry Mice were deeply anesthetized, and brains were processed as previously described [3]. By utilizing appropriately-labeled antibodies that attach selectively to their target antigens, it employs biochemical methods to scan distinct tissue components. IHC enables the high-resolution distribution and localization of cellular components within cells and within their appropriate histological context to be seen and documented [12].

Experiments

Experiment 1: Activation of the engram neurons in fear-conditioned F1 progeny as an expression of transgenerational memory

It was previously reported by Denny et al. that the cells reactivated during memory expression in the dentate gyrus (DG) and CA3 of mice's hippocampus are components of memory trace [6]. According to numerous previous studies on different organisms' traumatic experiences, fear-conditioning memory can be vertically transferred from parents (F0) to subsequent progeny (F1, F2, and so on). Therefore, we intend to test whether the engram cells of F0 mice can be reactivated in subsequent F1 progeny as an expression of the transgenerational fear-condition memory. Suppose the expression of the same engram cells is sufficient in F1 progeny. In that case, it can be concluded that these cells not only serve as components of memory trace in the F0 parent generation but also as the final form of transgenerational memory to certain stimuli, conferring the progeny the same memory of CFC.

In the experiment, we will first generate the F0 parents, let the memory trace form in their hippocampus, and use them to generate subsequent F1 progeny. ArcCreERT2 line will be generated through genetic engineering as the F0 parent generation. These F0 mice have both the ArcCreERT2 gene and the R26R-STOP-floxed-EYFP

gene, which serve as the basis for the later fluorescence and immunohistochemistry screening. Once the F0 mice reach adulthood (which takes approximately eight weeks), they will be administered tamoxifen and contextual fear-conditioning in Context A. They will be screened immediately for hippocampal neuron activation (marked by EYFP fluorescence).

In contrast, the others will be re-exposed in Context A after five days and re-administered contextual fear-conditioning to screen for another set of neuron activation. The overlapping neurons between these two sets of activated neurons will be the engram neurons. After the engram neurons are determined, the remaining F0 mice will be mated to generate F1 progeny.

The F1 progeny will be immediately screened for hippocampal neuron activation and compared against the engram neurons of their F0 parents to verify whether the memory trace is transferred trans-generationally through these cells.

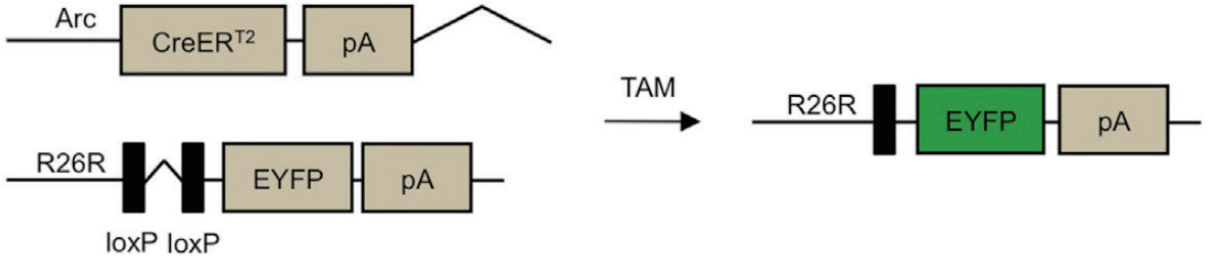

Figure 4. Inserting TAM into ArcCreERT2 x R26R-STOP-floxed-EYFP mice leads to an indelible EYFP label in the initially Arc+-activated cell [1].

Experiment 2: Transmission of the engram neurons to F2 mice

According to the report of Dias and Ressler in 2014, the olfactory experience of F0 parental mice can be vertically transferred to F2 generation. We thereby want to verify if the F2 inheritance still takes advantage of the engram neurons in the hippocampus. If these engram cells continue to sufficiently express in the F2 generation, then it can be concluded that the engram neurons can store memory traces sustainably across generations.

In the experiment, the F1 mice will be mated to generate the F2 generation once their post-fear-condition screening validates the sufficient expression of engram neurons in their hippocampus. The F2 generation will be fostered in the same manner as their F1 and F0 ancestors. As they reach adulthood after about eight weeks, they will be administered identical CFC to screen for the activation of hippocampal neurons. These activated neurons will then be compared with the F0 and F1 engram neurons, verifying whether the inheritance of the memory trace across two generations is achieved.

Expectation

When both EYFP+ and Arc+ are expressed, the neuron cells are expected to carry transgenerational memory and exhibit a yellow color [6]. However, suppose only the F0 cells exhibit yellow fluorescence and F1 cells exhibit green. In that case, it suggests that the response to the fear condition is not due to transgenerational memory stored in the CA3 region of the hippocampus that can be transmitted across generations. [6]

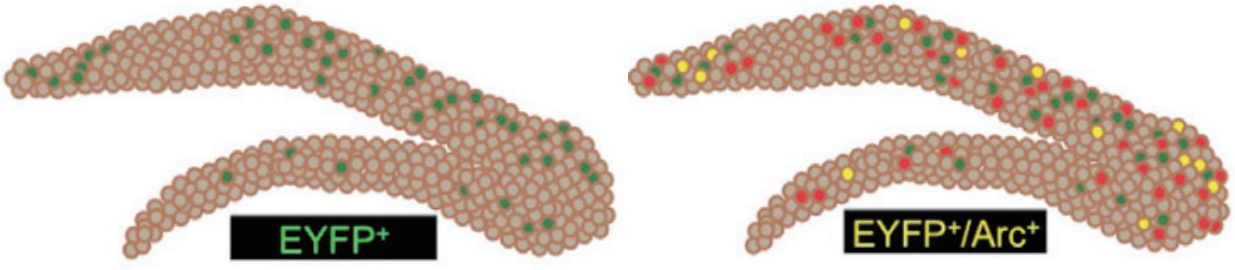

Figure 5. This image shows the colors exhibited when the EYFP+ gene is expressed and when both EYFP+ and Arc+ are expressed [1].

Conclusion

Let us recall the two experiments mentioned in the previous section. Experiment 1 was designed to measure whether the responses resulting from CFC used on the F0 generation were transmitted intergenerationally to the F1 generation by observing the number of activated storage memory neurons. Experiment 2 was designed to further observe on the F2 generation whether CFC affected them, even though they did not experience CFC personally. In summary, If the experiment results in yellow fluorescence in both the F1 and F2 generations as we expect, we can prove that memory can be stored in the CA3 region of the hippocampus and can be passed on through intergenerational transmission.

However, there are still some limitations in our experimental design; first, we only tested the number of memory neurons activated within two generations, and we did not go further to test it in three or four generations, so we could not confirm whether this transgenerational inheritance of memories could be inherited further down the line. Second, we did not set a specific number of neurons that indicated successfully transferred the memory, so we could not be very specific about the percentage of neurons that exhibited a yellow color to future generations of mice that would inherit this part of the stimulus-induced memory. Third, we did not measure the startle response in mice at the behavioral level, as mentioned in the previous paper, fear-potentiated startle.

These findings may be connected to the current theory that CA3 is impacted by the activity of the whole DG, which is modulated by young adult-born neurons [1]. Furthermore, these investigations might be useful in identifying memory declines that frequently go hand in hand with both healthy aging and age-related illnesses like Alzheimer's disease.

References

[1] Dias, B. G., & Ressler, K. J. (2014). Parental olfactory experience influences behavior and neural structure in subsequent generations. Nature Neuroscience, 17(1), 89–96. https://doi.org/10.1038/nn.3594

[2] Fitz-James, M. H., & Cavalli, G. (2022). Molecular mechanisms of transgenerational epigenetic inheritance. Nature reviews. Genetics, 23(6), 325–341. https://doi.org/10.1038/s41576-021-00438-5

[3] Zhu, H., Ding, G., Liu, X., & Huang, H. (2023). Developmental origins of diabetes mellitus: Environmental epigenomics and emerging patterns. Journal of diabetes, 15(7), 569–582. https://doi.org/10.1111/1753-0407.13403

[4] Nilsson EE, Sadler-Riggleman I, Skinner MK. Environmentally induced epigenetic transgenerational inheritance of disease. Environ Epigenet 2018;4: dvy016.

[5] Krabbe, S., Gründemann, J., & Lüthi, A. (2018). Amygdala Inhibitory Circuits Regulate Associative Fear Conditioning. Biological psychiatry, 83(10), 800–809. https://doi.org/10.1016/j.biopsych.2017.10.006

[6] Denny, C. A., Kheirbek, M. A., Alba, E. L., Tanaka, K. F., Brachman, R. A., Laughman, K. B., Tomm, N. K., Turi, G. F., Losonczy, A., & Hen, R. (2014). Hippocampal memory traces are differentially modulated by experience, time, and adult neurogenesis. Neuron, 83(1), 189–201. https://doi.org/10.1016/j.neuron.2014.05.018

[7] Casier, K., Boivin, A., Carré, C., & Teysset, L. (2019). Environmentally-Induced Transgenerational Epigenetic Inheritance: Implication of PIWI Interacting RNAs. Cells, 8(9), 1108. https://doi.org/10.3390/cells8091108

[8] Rose, N. R. & Klose, R. J. Understanding the relationship between DNA methylation and histone lysine methylation. Biochim. Biophys. Acta - Gene Regul. Mech. 1839, 1362–1372 (2014).

[9] Czech, B., Munafò, M., Ciabrelli, F., Eastwood, E. L., Fabry, M. H., Kneuss, E., & Hannon, G. J. (2018). piRNA-Guided Genome Defense: From Biogenesis to Silencing. Annual review of genetics, 52, 131–157. https://doi.org/10.1146/annurev-genet-120417-031441

[10] Luteijn, M. J., & Ketting, R. F. (2013). PIWI-interacting RNAs: from generation to transgenerational epigenetics. Nature reviews. Genetics, 14(8), 523–534. https://doi.org/10.1038/nrg3495

[11] Wiltgen, B. J., Sanders, M. J., Anagnostaras, S. G., Sage, J. R., & Fanselow, M. S. (2006). Context fear learning in the absence of the hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience, 26(20), 5484–5491. https://doi.org/10.1523/JNEUROSCI.2685-05.2006

[12] Liu, P. (2021, October). Sex Hormone and Sex Hormone Receptors Modulation of Exercise-induced Neurogenesis. In 2021 6th International Conference on Modern Management and Education Technology (MMET 2021) (pp. 543-548). Atlantis Press.