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Experimental Design: The Effect of Molecular Structure on the Inheritance of Transgenerational Memory

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Abstract: It is found that the olfactory experience of the parents could influence the behaviors and the structure of neuroanatomy. Transgenerational inheritance has been found in multiple species including animals and plants. The research in 2014 found that the epigenetic marks in the sperm of mice parent could be the mechanism of passing the transgenerational memory to the next generation, in which certain odor-responsive locus, Olfr151, has lower methylation level. However, it remains unclear that if Olfr151 is the only locus that is responsive for the inheritance of odor-conditioned transgenerational memory. Besides, there could be other mechanisms that can contribute to the inheritance of transgenerational memory. We designed this set of experiments to test if the transgenerational memory would be lost after restoring the methylation level of Olfr151 locus, and we will further research on the effect of molecular structure on the inheritance of transgenerational memory.

Keywords: Transgenerational memory, Transgenerational inheritance, Neuroanatomy

1. Introduction

Organisms are always facing the challenge of receiving and responding to the environmental stimuli, which requires them to draw on resources and avoid the harms. There are many ways that the responses of organisms can be influenced and an important one is the experience of their parents. According to research in 2014, *Parental olfactory experience influences behavior and neural structure in subsequent generations*, during which parents are conditioned with electric shocks and specific odor, the offsprings show changes in behaviors, neuroanatomical structures, and epigenetic marks corresponding to the order that their parents are conditioned with, which means that the environmental information received by parents could be inherited by the subsequent generations. Transgenerational memory can help the offsprings better detect, respond, and adapt to different environmental stimuli like shortage of food resources, changes of temperature, harmful odors and toxins. For example, when the Caenorhabditis elegans are exposed to harmful pathogen, they will produce the pathogen-induced diapause, which allows them to avoid the pathogen and evade infection, this could be inherited by next few generations of their offsprings. The phenomenon of transgenerational memory is not only found in animals but also in plants, while the mechanisms are kind of different. The epigenetic marks edited in animal germline cells are always erased during the meiosis, however, in plant cells, the epigenetic marks can be transferred to germline cells of offsprings, overcoming the easing and resetting process during meiosis, this could help plants to survive from conditions like drought However, it is also found that transgenerational inheritance could also lead to diseases. For example, the offsprings of pregnant mice that are exposed to endocrine disrupting

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chemicals could be found having obesity, which is due to different level of DNA methylation and RNA modification. The effect of transgenerational memory is also found in human. For example, there could be chemical coating on the chromosomes of survivors from the Holocaust, which not only affect themselves but can also lead to increased stress among their children. Transgenerational memory is known to be regulated by DNA methylations, histone modifications, and non-coding RNAs in the germ cells. The researchers found that hypomethylation in odor-responsive locus could affect the expression of odor-responsive gene and the number of odorant receptors.

Similar phenomenon of transgenerational memory is also found in other species. According to a in 2019 by Moore, Kaletsky and Murphy, it is discovered that worms can pass on transgenerational memory to the next few generations. When the parent worms are exposed to harmful pathogen, they will gain part of the piRNAs, which can be inherited by their offsprings. As a result, the offsprings of the next four generations will be able to recognize the pathogen and avoid the exposure of pathogen. Even though this transgenerational memory is lost in the fifth generation, this mechanism still helps the offspring to better survive.

The mechanisms of transgenerational memory in mice and worms could be different. In the case of worms, the transfer of memory is achieved by virus-like particles, which is more like a transfer of gene. However, in the case of mice, it could be that the stimuli applied to the parent alter the epigenetic marks in the gametes, which may then influence the neural structure of their offsprings.

Though the mechanism of inheritance of transgenerational memory if different, it is similar that transgenerational memories produced by environmental stimuli could only be inherited by a few generations, the effect of transgenerational memory could be reduced and even removed generation by generation. In this experiment, we are focusing on the effect of epigenetic marks on transgenerational memory, our main thinking and method is using CRISPR technique to restore the methylation of certain locus in the spermatocytes of parent to the normal level, and then we would observe the behaviors and molecular structure of epigenetic marks of the offsprings. Also, there's a limitation that former research only focused on and measured the methylation level of a certain odor-responsive locus, and there could be other loci that influence the traits of offsprings. We could hypothesize that epigenetic marks are crucial for transgenerational memory inheritance and the re-methylation process would remove the epigenetic marks, then restore the behaviors and neuroanatomy of offsprings.

2. Methods

In our experiments, we will use mice as experimental animals and fear conditioning as the method of producing transgenerational memory. The experimental mice will be conditioned with electric shock and different odor, which is acetophenone and propanol. And we would introduce CRISPR-dCas9-DNMT3a, which is a system of DNA methyltransferase that can increase the methylation level of a certain locus and inhibit the transcription and expression.

We will use two different kinds of mice, C57Bl/6J mice and M71-LacZ transgenic mice. Both of them will be conditioned, the former one will be used to make observation on behaviors, and the later one will be used to show the difference in neuroanatomy of odor sensitive neurons in the main olfactory bulb.

In this set of experiments, there will be two measurements related to the odorant sensibility of the mice. Firstly, when the mice are conditioned with odor and electric shock, not only will they perform a startle in motion when they are shocked, but they will also show the startle when they smell the odor that they are conditioned with, which is the odor potentiated startle as known as OPS. When a higher percentage of odor potentiated startle is observed on mice, it means that the mice have higher sensibility of the odor that they are conditioned with. Secondly, we will focus on the size of glomeruli that is responsive for the odor that the mice are conditioned with and the area of certain olfactory

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bulb. If the glomerulus area and the number of odor responsive neurons are larger in certain group of mice, it means that this group mice are more sensitive to the odor that they are conditioned with.

2.1. Experiment 1

In the first experiment, we will focus on fear conditioning the parent mice using electric shock with acetophenone and propanol. We will use sexually inexperienced and odor naive male mice as the F0 in the experiment, which will be fear conditioned and used to produce later generations of offspring mice. We will divide sexually inexperienced and odor naïve male mice into three groups. The first group of mice will be conditioned with acetophenone, which will be named F0-Ace. The second group will be conditioned with propanol, which is F0-Prop, and the last group of mice will be left in their home cage untreated, which is F0-Home, and this group of untreated mice will be used as negative control in the experiment. After this process, we are expecting that the mice in F0-Ace group will show increased odor potentiated startle to acetophenone than other two groups, and mice in F0-Prop will show increased OPS to propanol than other two groups, while the mice in F0-Home, which is the control group, will not show significant odor potentiated startle to neither acetophenone nor propanol. After performing fear-conditioning on the first and second group of mice and nothing on the third group of mice, all the mice will be mated with sexually inexperienced female mice. All the male offsprings of the parent mice will be used in the experiment. The offsprings of the mice in the F0-Ace group will be F1-Ace; the offsprings of the F0-Prop group of mice will be F1-Prop; and the offspring produced by the mice control group will be called F1-Home.

The observation of behaviors will be done on the C57Bl/6J mice. In the first part of the experiment, three groups of mice in F1 generation, F1-Ace-C57, F1-Prop-C57, and F1-Home-C57 will be exposed to the odor of acetophenone separately, and we will observe and record the percentages of odor potentiated startle of the three different groups. We are expecting that the mice in group F1-Ace-C57 will perform higher percentage of odor potentiated startle than the mice in group F1-Prop-C57 and F1-Home-C57, which means that F1-Ace-C57 mice are more sensitive to acetophenone than other two groups. Secondly, we will expose each group of mice to propanol and record the percentage of odor potentiated startle, and we are expecting that F1-Prop-C57 mice will show increased percentage odor potentiated startle than other two groups of mice, which means that they are more sensitive to propanol. In both parts of this experiment, the mice in F1-Home-C57 are expected to show similarly low level of percentage of odor potentiated startle. If the result of experiment 1 matches with our expectation, we can conclude that the offsprings of odor conditioned parent mice will show higher percentage of odor potentiated, which means a higher sensibility, to the odor that their parents are conditioned with.

The measurement of neuroanatomy will be done on the M71-LacZ transgenic mice in F1 generation. We will use the size of acetophenone responding glomeruli as a measurement of sensibility, if a group of mice are observed to have large size of acetophenone responding glomeruli, they will be considered to be more sensitive to acetophenone. We are expecting to find a larger number of acetophenone responsive neurons and a larger dorsal and medial area of acetophenone responding glomerulus area in the mice of F1-Ace-M71 group than that of other two groups, which means that offsprings of mice that are conditioned with acetophenone is more sensitive to acetophenone in neuroanatomical level than the mice in other two groups.

This part of the experiment is also a proof that the stimuli that applied to the parent generation will create transgenerational memory that can be passed on to the next generation.

After testing the difference of sensitivity of odors in different groups of mice, we will next exam the structure at molecular level. It is known that the level of methylation at certain locus can influence the transcription and expression of corresponding gene. If the locus has a higher level of methylation, the rate of transcription of this locus will be reduced, and the expression of gene that is related to this

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locus will be inhibited. We will perform bisulfite sequence to measure the level of methylation at Olfr151, which is a locus that is responsive for the odorant receptors for acetophenone and will affect the sensitivity of mice to acetophenone, in the sperms of F0 generation mice. Bisulfite sequence will turn all the C bases that are not methylated into U bases, and the bases that are methylated will remain unchanged, which will show the levels of methylation of different sites in certain locus. In this part of the experiment, we are expecting to observe hypomethylation at Olfr151 locus in the sperms of F0-Ace group of mice, and the methylation level of Olfr151 locus in the sperms of F0-Home and F0-Prop group will be higher than that of F1-Ace group, which is a normal level of methylation. If this part of the experiment show a similar result as what we are expecting, we can conclude that the stimuli received by parents will alter the epigenetic marks that can be transferred to their offsprings via gametes.

2.2. Experiment 2

In the second experiment, we will introduce CRISPR-dCas9-DNMT3a to edit the methylation level at the acetophenone-responsive locus Olfr151. CRISPR-dCas9-DNMT3s is a system of enzymes that can increase the level of methylation of certain locus. We use this enzyme to increase the methylation level at Olfr151 locus in the sperms of F0-Ace group mice, and after the application of CRISPR-dCas9-DNMT3, the methylation level of Olfr151 locus will be restored to the normal level, which should be similar to the methylation level of the mice in group F0-Home and F0-Prop. The CRISPR technique will be applied to the spermatocytes of F0-Ace mice, which will restore the methylation level, and after which we will perform in vitro fertilization using the edited sperm, which will produce F1-Ace-IVF and F1-Home-IVF mice.

After the production of IVF generation, we will expose each F1-Ace-IVF and F1-Home-IVF mice to the odor of acetophenone, and we would observe and record the percentages of odor potentiated startle for each group of mice, which represent their sensitivity to acetophenone. We are expecting that the F1-Ace-IVF will show a percentage of odor potentiated startle that is similar to that of the F1-Home-IVF group, which means that the sensitivity of acetophenone of F1-Ace-IVF mice is returned to the normal level. We will also exam the area of dorsal and medial bulb of F1-Ace-IVF mice and F1-Home-IVF mice, and we are expecting that the mice in the two groups will show similar size of dorsal and medial bulb as well as similar number of acetophenone responding neurons, which means that the difference in neuroanatomy is also recovered. If the second experiment get results that matches with our expectation, we would be able to conclude that after removing the epigenetic marks in sperms, the offsprings will not be able to inherit transgenerational memory from their parents.

3. Conclusion

However, it is still possible that after restoring the methylation level at Olfr151 locus to normal level, the F1-Ace-IVF group mice could show a higher percentage of odor potentiated startle than the mice in F1-Home-IVF group. And the number of acetophenone responding neurons and the area of medial and dorsal bulb could also be larger than that of F1-Home-IVF group. This could mean that even if the epigenetic mark at Olfr151 locus is removed, the transgenerational memory will still be inherited. In order to find out if there are other loci that is also responsive for the sensitivity of acetophenone, we are planning to apply bisulfite sequencing to other parts of mice genome. Bisulfite sequencing will turn all the cytosine into uracil except that the bases are methylated, so that we can find out the loci that have different level of methylation in F1-Ace-IVF mice compared to F1-Ace-Home mice. What's more, it is possible that the inheritance of transgenerational memory could be determine by other mechanisms including histone modification or non-coding RNAs.

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