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Transgenerational Inheritance of Odor-Specific Fear Memory: Engram Cell Analysis in F1 Offspring

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Abstract. This research illustrates the potential for studying transgenerational genetically modified mice. Male ArcCreERT2 x Ai14-tdTomato mice were used to label neurons activated during fear conditioning in which specific odors were paired with mild foot shock. Fathers were conditioned with Odor A, Odor B, or left unconditioned. F1 offspring were tested for memory recall using only Odor A and their brains processed to identify labeled neurons in the hippocampus. Two outcomes were possible: the number of engram neurons for Odor A would be increased in the F1 offspring of Odor A-conditioned fathers relative to the others, suggesting inheritance, or all offspring would have similar engram numbers, suggesting no effect across generations. This study contributes to our understanding of how experience-dependent memories may be passed on to subsequent generations and highlights the power of sophisticated genetic techniques in neuroscience.

Keywords: transgenerational inheritance, fear memory, engram cells, ArcCreERT2, Ai14-tdTomato, olfactory conditioning

1. Introduction

Memory allows an organism to adapt to past experiences by saving the information and by changing future behavior based on past events. Among all the different types of memory, fear memory is one of the most commonly used types to study fear associated behavior, because it can be readily induced and tested through natural behaviors such as freezing, for example. In addition, recent studies have indicated that personal experiences not only affect individual directly exposed but also their descendants, meaning previous conditions can also affect brain and behavior of following generations possibly through epigenetic alteration [1,2]. However, neural components of these transgenerational effects remain unknown.

Odor cues are very useful tools for understanding of conditioning in deer since they can be easily controlled and constantly paired with aversive stimulation. When specific odors are presented with mild foot shock, only discrete neural circuits involved in fear memory are activated. To investigate neural circuits at a cellular level in these studies AcreCreERT2 X Ai14td-Tomato mouse model was used. In this animal model

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2. Test method

CreERT2 recombinase is expressed under Arc promoter which become active in neurons after being stimulated by certain conditions therefore CreERT2 can be functional only if tamoxifen is present.

Therefore, labeling can be controlled temporally (only at desirable times) and conditions are met. Upon activation, CreRET2 will excise STOP sequence from Ai14 reporter gene leading to expression of red fluorescent protein tdTomato into neurons that were activated during fear conditioning [3]. Therefore, a durable and precise method of tracking neurons which were involved at time of formation of the memories could be achieved.

To summarize, with this research we investigated if memories that fathers form at time of conditioning change brain activity in hippocampus and behavior in offspring through inheritance of the same odorant. Male mice were conditioned with either Odor A, acetophenone, or Odor B, propanol. While all offspring were later tested with Odor A to assess potential transgenerational effects. The experiment test whether paternal exposure to an odor-shock association alters neural and behavioral responses in the F1 generation. It is hypothesized that offspring of odor A-conditioned father will exhibit a higher number of tdTomato-labeled hippocampal neurons when exposed to odor A, indicating inherited odor-specific fear memory. This work aims to clarify how experience-dependent changes in one generation may shape neural circuits in the next through possible epigenetic mechanisms [2,4].

Father mice were divided into three groups, with five animals per groups. The first group was conditioned with odor A, a 10% acetophenone paired with a o.5 mA foot shock, the second group with odor B, 1% 1-propanol paired with the same foot shock, and the third group served as unconditioned controls with no odor shock paring. Conditioning occurred over three consecutive days with 24 hours intervals. During each session, the odor was presented for 30 seconds, followed by a 2 second foot shock. This procedure consistently activated fear-related variability [5]. F1 offspring were tested exclusively with odor A to evaluate potential odor-specific inheritance of fear memory. They underwent a learning phase identical to that of the father, with odor A presentation followed by a 2 second foot shock across three consecutive sessions. Three days following the final session, F1 offspring were exposed to odor A in a recall test and freezing behavior was measured as an index of fear memory retrieval. To facilitate behavioral interpretation, a sub experiment was carried out in which distinct groups of F1 offspring were either exposed to odor A alone or received a short exposure to control for nonspecific stress or fear and also confirm the odor-specific inheritance.

Following behavioral testing, F1 mice were deeply anesthetized and perfused transcranial with phosphate-buffered saline followed by 4% paraformaldehyde for tissue fixation. Brains were quickly removed and postfixed to retain tissue structure and fluorescent labeling. Coronal sections from the hippocampus were cut using a vibratome and mounted on slides for imaging. Confocal microscope was used to image tdTomato-labeled cells at high resolution, allowing detailed mapping of cells activated during learning and recall. The combination of prompt tissue processing, precise sectioning, and sensitive imaging provided an effective method for assessing hippocampal engram cells in the offspring.

3. Conclusion

There are two possible outcomes for F1 offspring that would implicate differential transgenerational transmission of odor-specific fear memory. In one scenario, F1-Ace offspring from the fathers conditioned with odor A will have a greater number of tdTomato labeled hippocampal neurons as

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compared to F1-Prop offspring as well as unconditioned controls. An increase would indicate that paternal experience influences hippocampal neural architecture in an odor specific manner. Increased labeling would indicate that odor specific fear circuitry in the offspring are more strongly activated by exposure to odor A, perhaps reflecting inherited epigenetic changes or other heritable modulations of neural response. Correspondingly, these animals would show increased freezing in recall testing paralleling these molecular changes. These results would provide behavioral support for structural changes.

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