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13. ABSTRACT (Maximum 200 Words)

Learning disabilities severely deteriorate the life of many NF1 children by limiting their academic achievement, higher education and career choice. However, the pathogenic process for NF1-associated learning disabilities has not been fully understood and an effective therapy is not available. This study wasproposed to identify genes that are dysregulated in the hippocampus of the $Nf1^{+/-}$ mouse model by DNA microarray analysis. Characterization of these NF1-affected genes will dramatically improve our understanding of the molecularpathogenesis underlying NF1-associated learning deficits. During the first year of the project, we have (i) established an NF1 mouse colony to generate $NF1^{+/-}$ mice used in this study, (ii) purified RNAs from the NF1+/- and wild-type hippocampus and (iii)performed LTP experiments on NF1+/- and wild-type hippocampal slices to prepare slices after LTP induction for RNA purifications. These progresses lay down a firm foundation for us to complete the proposed microarray experiments in the next two years.

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Introduction

Learning disabilities severely deteriorate the life of many NF1 children by limiting their academic achievement, higher education and career choice (1). However, the pathogenic process for NF1-associated learning disabilities has not been fully understood and an effective therapy is not available. Drs Silva's and Zhong's laboratories have demonstrated that *Nf1* mutations lead to the development of learning deficits in mouse and *Drosophila*, respectively (2-4). Their work suggests that *Nf1* mutations cause learning deficits by disturbing the Ras/MAPK and/or cAMP signaling. Despite these significant progresses, NF1-affected downstream genes that directly contribute to deficits in synaptic plasticity and learning are largely unknown. We proposed to identify genes that are dysregulated in the hippocampus of the $Nf1^{+/-}$ mouse model. Characterization of these NF1-affected genes will dramatically improve our understanding of the molecular pathogenesis underlying NF1-associated learning deficits.

Body

During the first year of this project, we focused on following research activities outlined in the Statement of Work:

1. Establish a $NF1^{+/-}$ mouse colony (Task 1). As the first step of this project, we have to establish and maintain an NF1 mouse colony to produce $NF1^{+/-}$ mice in our laboratory. To this end, we obtained breeding pairs ($NF1^{+/-}$ x $NF1^{+/+}$) from the laboratory of Dr. Tyler Jacks, who generated the NF+/- mouse model by gene targeting technology (5). Unfortunately, we encountered a great difficulty to establish a colony from these breeding pairs since they failed to produce offspring. Later, Dr. Jacks' lab informed us that they also experienced the same difficulty at that time period and suggested us to obtain new breeding pairs from NIH. We successfully established an $NF1^{+/-}$ mouse colony from the NIH breeding pairs. We also established a PCR approach to genotype the offspring from this colony to identify $NF1^{+/-}$ mice. We recently were able to generate enough number of $NF1^{+/-}$ mice to start experiments. The difficulty we experienced to establish an NF1 mouse colony significantly hampered our research in the first year.
2. Purify RNA from the hippocampus of $NF1^{+/-}$ and wild-type mice (Task 1). Using the $NF1^{+/-}$ and their wild type littermates, we purified RNAs from their hippocampi. To successfully identify genes that are not only dysregulated in the $NF1^{+/-}$ hippocampus but also are contribute to the development of learning disabilities of the NF1 mice, we realized that it is important to determine the behavioral conditions under which to collect hippocampal samples for RNA purification. We set up a behavioral test of context discrimination that was showed previously to differentiate the hippocampus-dependent learning abilities of the wild-type and NF1+/- mice (6). Consistent with previous findings (6), our initial results indicate that $NF1^{+/-}$ mice show impairments in this learning paradigm after 1 day training. However, as suggested by previous studies (2, 3),

this learning deficiency may be overcome by overtraining of multiple days or by reducing Ras activities. To better address the research topic of characterization of genes that are affected by NF1 mutation and associated with NF1 learning disabilities as proposed, we recommend changes to include hippocampi from: (i). without training, (ii) 1 day of training, (iii) 2 days of training, (iv) 3 days of training, (v) without training but treated with lavostatin (suggested by Dr. Alcino Silva to rescue $NF1^{+/-}$-associated learning disabilities by reducing Ras activities, basing on his submitted work), (vi) 1 day of training plus lavostatin treatments, (vii) 2 days of training plus lovastatin treatments and (viii) 3 days of training plus lavostatin treatments. In this way, we will be able to correlate NF1-dysregulated genes with learning behaviors when we perform analysis of microarray data, and identify those NF1-dysregulated genes that are most closely associated with learning disabilities. Currently, we have purified RNAs from the group (i) hippocampus as described in the original proposal.

3. Perform LTP experiments on wild-type control and NF1 hippocampal slices (Task 2). To identify genes that are dysregulated by the NF1 mutation during LTP expression, we proposed to first compare the tetanus-induced LTP expressed in the wild-type and $NF1^{+/-}$ hippocampal slices. We prepared hippocampal slices from adult mice and induced LTP with 4 trains of 100Hz stimulations spaced by 30 seconds. Currently, we can successfully induce LTP in the $NF1^{+/-}$ hippocampal slices. Consistent with previous studies from Dr. Silva's laboratory (3), our preliminary data appear to suggest a decreased magnitude of LTP in the $NF1^{+/-}$ hippocampal slices. We are currently collecting hippocampal slices after LTP induction to purify RNA for microarray analysis.

Key Research Accomplishments

1. Establishment of the NF1mouse colony.
2. Establishment of a method to genotype $NF1^{+/-}$ mice.
3. Purification of RNA from the wild-type and $NF1^{+/-}$ hippocampus.
4. Induction of LTP in wild-type and NF1+/- hippocampal slices by tetanic stimulations.

Reportable Outcomes

We do not have manuscripts from this research yet, but expect to have when the project is completed. By performing the described research work, two trainees (one postdoctoral fellow and one young research technician) gained experiences in NF1 research. A postdoctoral researcher in the lab is interested in applying for a fellowship to carry out NF1-related research using the NF1 mice we have in the lab.

Conclusions

In summary, we have successfully established the NF1 mouse colony to generate NF1+/- mice for our research. Although an unexpected difficulty was encountered during the course of breeding, we made significant progress in consistent with the work described in the Statement of Work. We purified hippocampal RNAs from the wild-type and the $NF1^{+/-}$ mice. As outlined in section 2 in BODY, we recommend minor changes in collecting hippocampi to include more groups of animals with different training conditions, which will render us power in analysis of microarray data in the future to identify genes that are not only dysregulated in the $NF1^{+/-}$ hippocampus but also show close correlations with learning disabilities of the $NF1^{+/-}$ mice. We also performed LTP experiments on $NF1^{+/-}$ hippocampal slices, which will allow us to collect tissues after LTP induction for RNA purifications. These progresses have paved the way for us to perform microarray analysis in the next two years.

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