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Interfering the inter-spine competition using cadherin/catenin complex as a potential treatment in ASDs

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Abstract. Autism, a type of genetic disease, was found to be related to various gene mutations like SHANK 3 and SHANK2. The direct results are deficits in spine pruning in early childhood while pruning's frequency is lower than usual. In this essay, we investigate a potential treatment for genetic disease by adjusting a type of complex—cadherin/catenin complex, which is a complex that might be able to reverse the effect of spine pruning loss. A specific experiment was designed to fulfill our purpose. The significance of this work is that it sheds light on a possible treatment for an innate genetic disease. However, all of the methods mentioned are theoretical, requiring further implementation.

Keywords: Autism, Cadherin/catenin complex, spine pruning, experiment.

1. Introduction

Autism spectrum disorder, also called ASD, is a type of neurodevelopmental disease that is marked by features: impaired social behaviors and difficulties in communication. ASD is a kind of genetic disorder that multiple susceptibility genes can cause. For instance, mutations in SHANK 3 and SHANK 2 all-cause neurodevelopmental deficits and autistic symptoms. Moreover, a mutation associated with tuberous sclerosis also shows autistic symptoms [1]. ASD's genetic heterogeneity makes it difficult to cure from its source.

It was found that there has been increased spine density in the frontal, temporal, and parietal lobes of ASD patients' brains [2], while the mechanism under the spine pathology and how can growing spines lead to ASD-like symptoms are still not specified. The high spine density is related to reduced spine pruning in layer V pyramidal neurons in the temporal lobe and impaired autophagy [3].

Therefore, it was figured that if the cadherin/catenin complex, a regulatory protein located in both presynaptic and postsynaptic membranes, can be manipulated genetically by enhancing its expression, it could offset the spine pruning defects in ASD patients. Nevertheless, the uncertainty of whether the increased expression of the cadherin/catenin complex can prune the correct spines becomes an issue.

2. Literature Review

2.1. Development trends of humans

Formation and elimination of dendritic spines consist of the process of synaptic development [4]. In early childhood, spine density would increase significantly since spine formation outpaces spine pruning.

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Then elimination gradually exceeds the formation, leading to a sharp decline in spine density. This process allows the selection of synapses and refinement of neural circuits [5]. The choice of spines being strengthened is dependent on their function and whether their parts are still crucial or not. Climbing fiber in Purkinje cells effectively exemplifies such a dynamic [6]. One central hypothesis states that the shrinkage and extension of the spines are strongly correlated with calcium ion concentration and that its differentiated amount of rises and drop affect spine pruning and elongation [7].

2.2. Characterization of Autism Spectrum Disorder

ASD is defined as a pervasive spectrum disorder influencing people's social interaction. It is linked to various genes, including SHANK3, NRXN1, CNTNAP2, etc. While the different correlated genes encode for several structures ranging from extracellular binding ligand and cytoplasmic binding partners to proteins in the Golgi body, it is impossible to solve the conditions simply by altering a single gene [1]. This essay focuses on SHANK mutation in ASD patients. SHANK3 mutated macaques are found to display ASD-like behaviors, including repetitive behavior, reduced social interaction, and enhanced anxiety [8]. fMRI images of those macaques demonstrate reduced connectivity between the prefrontal cortex and other brain regions and altered connectivity in regions correlated with cognition and communication. SHANK3 mutation plays a vital role in ASD and significantly alters people's behaviors.

However, in ASD patients, the spine density does not decline during late childhood and adolescence.

Specifically, researchers found using mouse models that the mTOR pathway, which is closely associated with macroautophagy, is inhibited in ASD present individuals. A direct result of such disruption is impairment in spine elimination and excess synaptic materials. Additionally, the mice that are deficient in macroautophagy and disrupted in the mTOR pathway show ASD like behaviors, which implies a link between ASD's functional abnormalities and macroautophagy dysfunction [9].

2.3. Mechanism of Cadherin-catenin Complex

Cadherin/Catenin complex located at pre and postsynaptic membrane, and able to regulate plasticity of neurons by activation of transduction and regulation of synapses [10]. Also, it is a mediator of spine pruning [11]. In detail, cadherins are transmembrane proteins that form hydrophilic interaction, thereby facilitating adhesion between synapses. Catenins bind to the tails of cadherins and actin cytoskeleton, which indicates its role in stabilizing the structure of synapses. Moreover, during synaptogenesis, cadherin-catenin complexes guide axonal and dendritic growth and establish synaptic connections with matching partners. All of these indicate how the complex influences synaptic specificity and selectivity. In addition, as their correlated pathways also influence gene expression and synaptic remodeling, caherin-catenin complex also potentially affects neural function and adaptation [12].

Cadherin-catenin complex forms a competition model when its cohesion is elevated, and photo-stimulating the axon with the expression of photosensory receptors can stabilize the contacting spines and eliminate the neighboring ones. It's discovered that during the postnatal period, neighboring spines tend to "compete" for cadherin-catenin complexes and help redistribute those complexes in order to eliminate the less active spines and strengthen the more active spines. This "competence" between spines is critical in the acceleration of the maturement of spines and shapes the neural circuit in brains [11]. After all, restricted expression patterns in catenin complexes are also found to be correlated with ASD and other mental disorders[13-15]. Therefore, it was figured that genetic modification of cadherin-catenin complexes in ASD patients might be helpful in alleviating or alter the ASD symptoms.

2.4. Ocular Dominance Shift

This can be examined utilizing ocular dominance columns, neurons in certain mammalian's visual cortexes that include preferential response to input. Ocular dominance columns are specialized areas that process information from each eye and influence neural development in the visual cortex. During critical periods, visual input largely affects neural connections, including spine pruning and elongation. An instance would be an infant with monocular eye closure in the first few months would have a reduced number of cells activated by close eyes and vice versa.[16]. It undergoes neuronal development by visual

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stimuli to eliminate and maintain spines in the visual cortex accordingly. In other words, its plasticity is completely based on visual input and experiences [17]. This trait makes it crucial for the conduct of the experiment, as it is able to be detected and make conclusions about how the complexes influence the neural circuits.

3. Experimental Procedure

There are mainly two experiments designed: ocular dominance columns shift in mice and social interaction with environment enrichment condition in the prefrontal cortex.

3.1. Ocular dominance columns shift in mice

The main purpose of this experiment is to determine whether the increased expression of the cadherin/catenin complex can lead to correct spine pruning. Three groups of mice will be set, with 20 mice in each group; each mouse will have approximately the same size. All the mice will have their left eyes covered with a transparent patch to create differential visual stimuli. Moreover, boxes with colorful and identical pictures contain all of the mice throughout the experiment to provide sufficient visual stimuli.

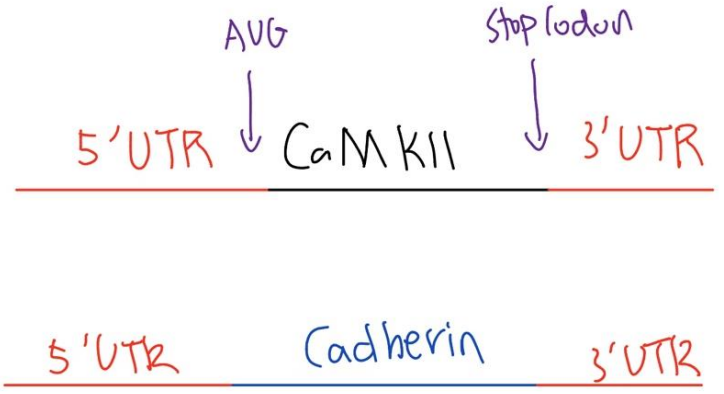

Figure 1. Mechanism of knockout.

Figure 1 illustrates the simple mechanism of how mRNA has been modified so that translation of CaMKII and cadherin/catenin complex would be associated to achieve the purpose. Before each mouse is observed, some will be first genetically modified that Shank 2 gene in the visual cortex will be knocked out. This is done to imitate the situation of an autism patient with spine pruning defect. Then, the mRNA region that encodes for cadherin or catenin will be bind to 3 prime UTR region of Cam Kinase II(CaMKII), an enzyme activated by increasing calcium ions and calmodulin to regulate several processes including neurotransmitter secretion. So, each time when CaMKII was translated, the translation of cadherin or catenin also increased.

After the genetic adjustment, rabies virus would be infected by inserting fluorescent protein into the axonal termini of left and right eye each with green fluorescent protein and tdTomato, a kind of red fluorescent protein, in order to better distinguish the image during the critical period.

The experimental group includes the modified expression of SHANK 2 gene and cadherin and catenin. Two control groups are set while one group being completely normal mice and another group are the mice with SHANK 2 mutation. After 40 days of neuronal development, the spine density and percentage of matured dendritic spines of each mouse would be analyzed by pair spine analysis, a method to detect the change by comparing a pair of spines.

The expectation of the result is that in the experimental group right eyes will show more spine pruning and therefore lower spine density than the right eye, just like the control group with normal mice. Because the adjustment of cadherin/catenin complex is able to offset the effect of our deliberate mutation. While another control group with only SHANK 2 mutation should show no significant

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difference even in the presence of different stimuli to the visual cortex due to its dysfunction of spine pruning and comparatively lower amount of cadherin/catenin complexes.

3.2. PFC social interaction with EE

Is the manipulation of cadherin and catenin able to eliminate the autistic symptoms by coordinating the inter-spine competition? In the next experiment, we utilize the same trans-genetic technique of mutating SHANK 2 gene but in prefrontal cortex since it is the region responsible for social activity. Moreover, green fluorescent protein will be attached to cadherin or catenin in this case, so we can ensure that the increased expression of cadherin and catenin has occurred in the genetically modified mice.

There are still three groups set each with 20 mice of identical size and weight. The experimental group include the modification of both cadherin/catenin complex and SHANK 2 gene. Negative control group are normal mice whereas positive control group are autistic mice with a normal level of cadherin/catenin complex.

In this experiment, EE condition is introduced to create more social stimuli rather than only putting a group of mice together. Environmental enrichment is achieved by mice toys that provide different social stimuli. Each group of mice would be put in such an environment also with other 20 normal mice for 40 days of the critical period. The result will be examined not only by analyzing the spine density and matured spines but also by behavior tests. The autistic symptoms of mice could be foretold by their response to novelty since normal mice tend to approach novelty. A three-chamber test will be carried out: the mice will be put in the middle of three chambers to observe their decision-making to determine if they have ASD or not.

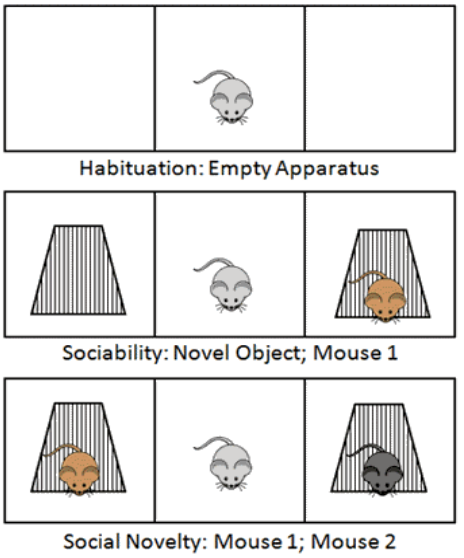

Figure 2. Illustration of the testing social behaviors on mice [10].

The prediction of the results would be that the experimental group will always have similar results to the negative control group. They should show similar spine density and percentage of matured spines, and similar behaviors is consistent with the hypothesis. The positive control group, however, would show distinctive results of higher density of spines and distinct social behavior (see Figure 2).

4. Conclusion

In summary, two experiments were designed respectively utilizing the same trans-genetic technique to either increase the cadherin/catenin complex expression or to knock out the SHANK 2 gene. In the experiment done in the visual cortex, it is mainly achieved to detect if the spine pruning caused by the cadherin/catenin complex would eliminate the spines correctly but not randomly while utilizing the traits

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of ocular dominance columns and setting two variables of genetic modification. The experiment conducted in PFC is more directly correlated with autism spectrum disorder by examining the relationship between autistic symptoms and the expression of the cadherin/catenin complex.

Surely some limitations lie within: first, humans have a more complex nervous system than mice, the results of the experiment may not be consistent in humans even if the hypothesis is proven correct; second, the two experiments are not conducted in the same region of the brain, uncertainties exist that there are other possible unknown mechanisms that make the whole model unpredictable; At last, the number of mice in trials might not be sufficient to be a strong database to support the hypothesis. In other words, coincidences could happen that lead to changed results.

The original purpose of this project is to provide a proper cure for ASDs. The future direction is contingent on the actual result. If the hypothesis is disproven, more researches and consummation need to be done. If the hypothesis is proven to be true, then this experiment could be conducted in primates which are more similar to humans. Nevertheless, even if all the experiments are successful, it is still not pragmatic to be a treatment for autism patients. Because all of the procedure includes genetic modification, which for now is not possible to do on humans. So, the most significant future direction is to instead investigate some drugs that are able to trigger a pathway causing the increased expression of cadherin or catenin.

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