Treatment for ASD patients with the cadherin and catenin complexes

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Abstract

The cadherin and catenin complexes are responsible for refinements and proper neural circuit formation during the critical period. Abnormal refinement causes deficiencies in spine pruning and neuronal disorders, including autism spectrum disorder. Autism spectrum disorder (ASD) is a developmental disorder caused by defects in the spine competition. An increase in the cadherin and catenin complex can simulate the synaptic signal transmission and increase competition to maintain average spine density. However, the proper neuronal function requires the elimination of unnecessary spines. Therefore, this paper designs an experiment using an ocular dominance column in mice to evaluate whether the cadherin and catenin complex can overcome the deficiencies in spine pruning for ASD mice and whether spine pruning is correct. Then, the cadherin and catenin complex is adopted on PFC in mice to evaluate the effect of correction on social behavior for ASD mice. This work proposes a better treatment for ASD patients using the cadherin and catenin complex.

Keywords: spine pruning, autism, cadherin and catenin complex, spine competition

1. Introduction

Precise neuronal connection formation and proper refinement are crucial for brain development [1]. A previous study suggested that spine density first significantly increases after birth, followed by a rapid net decrease caused by spine pruning, when spines on the peripheral dendrites compete for the limited neurotrophic factors, including the cadherin and catenin complex [2]. During brain maturation, neuroactivity drives spines to compete for the cadherin and catenin complex, redistributing the complex from less active spines to more active spines. The more active spines, being functionally important, are matured and grow in size, while the less active spines are eliminated or pruned. A recent study suggests that the cadherin and catenin complex is sufficient and necessary to maintain spine maturation and pruning, indicating that the defects in availability can cause disorders [3].

One disorder caused by deficiencies in spine pruning is autism spectrum disorder (ASD). Autism spectrum disorder is a pervasive developmental disorder affecting 1/44 of children in the US [4]. ASD patients face social interaction difficulties, impaired communication skills, and repetitive behaviours. Researchers have identified several mutated genes that inhibit mammalian target of rapamycin (mTOR) kinase and could lead to deficiencies in autophagy and spine pruning, including Atg7, SHANK2, SHANK3, etc [5,6]. Therefore, rapamycin is proposed as a treatment for autism by inhibiting mTOR molecules and maintaining normal spine pruning. However, there are several deficiencies with the use of rapamycin. Most importantly, the mTOR molecule is used in many brain regions and is responsible for protein synthesis, learning and memory, apart from autophagy [7]. Therefore, rapamycin can bring side effects by inhibiting protein synthesis. In addition, genes identified that could be mutated to cause spine pruning defects are all downstream factors. Therefore, a treatment specific to increase spine pruning and non-unique to specific gene mutation is required to treat ASD patients.

In contrast, the cadherin and catenin complex can be an ideal treatment for ASD patients. The competition-based model supports an inverse proportional relationship between spine size and density for normal neuronal development [3]. ASD patients, though, have deficiencies in spine pruning with normal spine maturation. It is demonstrated that the signals in synapses generated by the ASD patients are too weak to overcome the threshold. Therefore, increasing the cadherin and catenin complex in the synapses can increase the competition and overcome the threshold. The cadherin and catenin complex is also necessary and sufficient for spine competition, indicating that increasing the cadherin and catenin complex can be a possible way to increase spine competition and pruning. Compared with rapamycin, the cadherin and catenin complex is localized to the synapses, with no interference to other brain regions. In addition, the complex is highly specific to spine formation, eliminating the risk of introducing side effects.

However, whether the increase in spine pruning can overcome the autistic spine pruning defect is still worth examining. In addition, to maintain normal neuronal development, the cadherin and catenin complex need to

eliminate the unnecessary spines instead of randomly eliminating them. Labelling the axons in the PFC for mice is impossible, so the experiment is first conducted in the sensory cortex with ocular dominance column segregation [8]. Therefore, the cadherin and catenin complex is first used in mice with a Shank2 knockout in the sensory cortex for better evaluations. The ocular dominance column, stripes of neurons that deal with stimulation from one eye, can be used to examine the pruning process [9]. Using rabies virus allows labelling of the axons in both eyes separately. Then, the same process is adopted to the PFC and examines the correctness of spine pruning by social evaluations. Whether spine pruning is correct in the sensory cortex and PFC in autistic mice shows whether cadherin and catenin complex can be used in autism treatment.

2. Experiment

2.1 *Experiment I Cadherin and Catenin Complex in the mice visual cortex*

This experiment evaluates whether the cadherin and catenin complex can overcome spine pruning defects in the sensory cortex and result in normal spine pruning.

Three groups of male Long–Evans rats are used, each group containing twenty mice having a relatively similar size and weight, health condition and age around P20. The rats are placed individually in a box with a 12hr light-dark cycle with different colour pictures. The rats are placed separately in the colony room with free access to unlimited food.

Before the experiment, we applied a CRISPR–Cas9 strategy that targets exon 7 of the macaque, as shown in Table 1 below. SHANK2 gene is knocked out in the rats' visual cortex in the experimental and control groups I, but not control group II. The SHANK2 gene is responsible for spine maturation [10], so knocking out the SHANK2 gene in the sensory cortex simulates autistic symptoms in the sensory cortex, causing deficiencies in spine pruning and an increase in spine density. Rabies virus is used to insert the fluorescent gene into the left and right eye axons to observe the effect of spine pruning by labelling axons. The left eyes were dyed with GFP, and the right eyes were stained with TdTomato.

The cadherin and catenin complex are added to the synapses using the transgenic technique for the experimental group. The gene that codes for cadherin and catenin on the mRNA is attached to the CaMKII 3'UTR mRNA. Therefore, when the CaMKII is translated in the synapses, the mRNA coding for cadherin and catenin complex would also be translated to increase the amount of cadherin and catenin complex in the synapses. The left eyes of the three groups are covered with a transparent patch to interfere with their vision. Therefore, the visual stimulus received by the two eyes is slightly different, simulating the situations of the EE conditions in social interaction.

Table 1. Cadherin and Catenin Complex Experimental Design

Groups	Gene knocked out in the VC	Transgenic technique	Eyes covered
Experimental Group	Shank2	Cadherin and Catenin Complex	Yes
Control Group I	Shank2	None	Yes
Control Group II	none	None	Yes

Then, after 40 days of postnatal development, the three groups of mice received an intraperitoneal injection of 1.5 ml sodium pentobarbital (60 mg/kg). Anesthetized mice were then perfused intracardially, followed by a 10% (wt/vol) Formalin solution[11]. Then, we take out samples of the visual cortex postsynaptic neuron in the mice to examine the spine maturation and spine density in the left and right eyes and compare them under the microscope. The neurons responsible for the left and right eyes can be distinguished in different fluorescent colours. The labelled axons are compared in the three groups to see whether the spine pruning in the experimental group is correct or not.

2.2 *Experiment II PFC social interaction*

The following experiment is focused on the postsynaptic neurones in the Prefrontal cortex, which are responsible for social interaction [12]. Three groups of male Long–Evans rats are used, each group containing twenty mice having a relatively similar size and weight, health condition and age around P20, before the critical period.

Before the experiment, we also applied a CRISPR–Cas9 strategy that targets exon 7 of the macaque in the Prefrontal cortex to the experimental group and negative control group. This causes deficiencies in spine pruning in the PFC, leading to deficiencies in social interaction. The positive control group contains 20 normal mice without

genetic modification.

During the experiment, the three groups of mice are placed in Environmental Enrichment conditions with 20 normal mice, providing external social stimulus to 20 normal mice through 40 days of development, as shown in Table 2. Then, apply the same trans-genetic technique to the experimental group, only using CaMKII to increase the translation of cadherin and catenin; therefore, the proportion of cadherin and catenin complex increases in the PFC postsynaptic dendrite. In addition, the gene that codes for the GFP is added to the CaMKII mRNA so that the cadherin and catenin produced will be fluorescent in the spines, making it easier to evaluate the results.

Table2. PFC Social Interaction Experimental Design

Groups	Gene Mutation	Trans-genetic Tech	Environment Enrichment (EE)
Experimental Group	Shank2	Cadherin and Catenin Complex	Placed
Positive Control Group	None	None	Placed
Negative Control Group	Shank2	None	Placed

However, there is no way to evaluate the precision of spine pruning directly. Therefore, after 40 days of postnatal development, social evaluations are applied to the three groups of mice to examine whether they have autistic behaviours. The three-chamber test is adopted for the three groups of mice to evaluate their social preference. The novel sociability and social novelty tests are taken to calculate the preference index, which is used to determine the impairment in social interactions. In the sociability test, a decrease in preference index (the ratio of time sniffling other rats by non-social objects) is observed as impaired social interaction. For the social novelty test, the decrease in preference index (the ratio of time sniffling novel rats to familiar rats) is observed as impaired social interactions. Then, the mice were again deeply anaesthetised and killed by the Formalin solution, as in the previous experiment [11]. Then, we take out samples of postsynaptic spine tissue samples from the PFC in the mice to examine the spine maturation and spine density through imaging.

3. Overall Methodology

Measuring and comparing the spine density in the sensory cortex evaluates whether spine pruning can be corrected with cadherin and catenin complex for autistic mice in the sensory cortex. Normal spine pruning in the sensory cortex using the ocular dominance column could show that the cadherin and catenin complex can correct the deficit in spine pruning in the visual cortex. However, the main deficiencies in spine pruning for ASDs occur in the PFC. Therefore, the same method is adopted in the prefrontal cortex with EE conditions to see whether the cadherin and catenin complex can result in normal spine pruning for autistic mice and result in normal social interactions. Tests in the social preference index are used to determine the impairment in social interactions, a symptom of ASDs. The increase in spine pruning compared with the control group could reveal the correctness of spine pruning with cadherin and catenin complex in the PFC.

4. Results

4.1 *Ocular dominance shift*

If the experiment results are consistent with the hypothesis, the experimental group should have normal spine pruning, with the area of the ocular dominance column of the right eye larger than that of the left eye. The results of the control II group should be similar to the experimental group. For the control I group, the spine pruning of the left and right eye should be the same, with a similar area of ocular dominance column for the two eyes. This suggests that the cadherin and catenin complex increase correct spine pruning in the visual cortex.

4.2 *PFC*

If the results of the PFC are consistent with the hypothesis, the experimental group should have normal spine pruning and normal preference index, the same as the positive control group. The negative control group should have an increase in spine density compared with the experimental group, with a decreasing preference index. This suggests that the cadherin and catenin complex overcome the spine pruning deficits, resulting in normal spine pruning in the PFC.

4.3 *Limitations*

4.3.1 *Mice and human*

Differences between mice and humans are one of the limitations of the research. There are structural differences between mice and humans in the PFC [13]. In fact, the nervous system in humans is much more complicated than that of mice, so the treatment applied to mice may

not be effective in humans. For instance, some drugs treating cancer in mice cannot produce the same effects in humans. In addition, spine pruning in the PFC is different between human and non-human primates in the period, possibly caused by the increase in the importance of the prefrontal cortex in humans. However, considering that it is unethical and unapproachable to use humans as experimental targets and limitations in technique, the experiment can only be conducted on mice, with a risk of ineffectiveness for humans. In addition, the chemicals applied to mice may be toxic to humans.

4.3.2 *Number of mice*

In this experiment, only 20 rats were used in one group. The insufficient use of mice may cause the inaccuracy of the results. Therefore, if the results are consistent with the experiment, an increasing number of mice should be used to ensure the accuracy of the results and eliminate the possibility of producing random effects.

4.3.3 *Prefrontal cortex and sensory cortex*

The difference in structures between the prefrontal cortex and the sensory cortex is also a limitation of the experiment. In humans, the time of pruning is different between the sensory cortex and the prefrontal cortex, which may suggest a slightly different mechanism. Therefore, the cadherin and catenin complex may not produce the same results in the sensory cortex and the prefrontal cortex.

5. Conclusion

The previous experiments are conducted to evaluate whether the cadherin and catenin complex can be used in treating autism patients. Therefore, if the results are consistent with the hypothesis, more experiments should be done on primates, particularly on monkeys, which have more complicated nervous systems and similar structures to humans. In addition, drugs should be designed to apply such treatment to humans instead of using trans-genetic methods in treating patients. Somatic modification can be used to increase the expression of RNA responsible for the cadherin and catenin complex translation. If the results are not consistent with the experiment, the experiment should be consummated to find the problems when conducting the experiment and the logic deficits.

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