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**Disruption of DISC1 via knockout of NDEL1 stunts spine formation in the hippocampus region of mice**

1. **Introduction**

Schizophrenia is a chronic, debilitating mental disorder that currently affects around 1% of the world’s population. Symptoms vary throughout individuals, however, they are all categorized in about the same three categories: positive, negative, and cognitive. Examples of positive symptoms include hallucinations and delusions, while negative symptoms are more along the lines of social withdrawal and lack of motivation. Lastly, cognitive symptoms include impairment of memory and attention. Because of the wide, variety of symptoms, many patients have high rates of homelessness, violence, and suicide (World Health Organization). These prognoses seriously impact the economy and society - costing upwards of $62 billion dollars per year (Ellaithy et al., 2015). It is for this reason that the World Health Organization ranks this disorder among the top 10 causes of disability in the world (Ellaithy et al., 2015).

While schizophrenia is such a prevalent and debilitating mental illness to individuals and the population, most drug therapy aimed at treating schizophrenia does a very poor job. While typical antipsychotic drugs are effective against positive symptoms, they also demonstrate a limited efficacy against negative symptoms and cognitive impairments - which have been shown to contribute to functional impairment and predict poor prognosis (Ellaithy et al., 2015, Moreno et al., 2011). These drugs were introduced into clinical practice in the early 1900s and despite the increasing research on schizophrenia, they have not changed much in their chemical structure since then. The limitations of the presently available drugs underscore the need for identification of new antipsychotic compounds aiming at new molecular targets.

One such target, that has been heavily studied, is Disrupted-in-Schizophrenia-1, a gene-encoding protein that is identified as a genetic risk factor across a spectrum of psychiatric disorders. DISC1 is present at the intersection of several neurodevelopmental pathways and acts as a scaffold - binding a number of other proteins together, which have all been shown to be independent risk factors for major mental illnesses as well (Duan et al., 2007, Soares et al., 2011). Recent studies have suggested a link between DISC1 genotypes and elements of neurocognitive function (Duan et al., 2007). However, much about DISC1 is known and thus represents a challenge to drug target due to the absence of a solved structure (Soares et al., 2011). Thus, drug therapies involved with DISC1 would need to focus on modulate interaction of DISC1 with one of its many binding partners.

One of DISC1’s many binding partners includes Nuclear Distribution Element-like 1 (NDEL1). NDEL1 is a centrosomal protein that is involved in mitosis, neuronal migration, neuroplasticity, and neurogenesis during brain development (Burdick et al., 2008). Neural plasticity refers to the brain’s ability to make changes to itself throughout its lifetime. A recent study has demonstrated that NDEL1 expression is decreased in the hippocampus region of those suffering with schizophrenia (Burdick et al., 2008). This suggests that the plasticity of the brain, or it’s ability to adapt, can lead to changes in cognition and behavior. Cognitive deficits, such as those talked about above as a symptom of schizophrenia, may then be a result and consequence of deficits in neural plasticity (Voineskos et al., 2013). As well, an intact NDEL1-DISC1 interaction has been shown to be critical to multiple developmental processes such as neural outgrowth (Voineskos et al., 2013). Both of these aspects suggest the importance of NDEL1 and DISC1 in understanding a new aspect and relationship in schizophrenia.

While there have been some studies examining the relationship between NDEL1 and DISC1, much still has to be studied and understood. Does knockout of one protein affect the function of the other? How does the knockout of the protein lead to changes in plasticity and changes in the formation of dendrites in the brain? The purpose of this experiment is to answer similar questions by testing whether knockout of NDEL1 impacts the function of DISC1 in the spine formation of the hippocampus region.

1. **Experiment**

The aim of this experiment is to understand the relationship between the proteins NDEL1 and DISC1 in the development of the spine structure in the hippocampus region of the brain. Samples of the two proteins will be injected inside adult mice to gather and measure the dendritic spine growth and formation within the hippocampus region. In these samples, NDEL1 will be knocked out to test how it’s silence impacts the function of DISC1. I would expect that the level of spine formation within the hippocampus be similar to that with a DISC1 knockout, i.e. under conditions that would negatively impact the cell.

* 1. **Co-immunoprecipitation**

Co-immunoprecipitation is a popular technique used to protein-protein interactions by using target protein-specific antibodies to capture and pull down proteins that are bound to a specific target protein. This method was employed by Moreno et al. (2012) to detect mGlu2 with an anti-HA antibody by capturing 5-HT2A with an anti-c-Myc antibody. As Figure 1 illustrates, they first incubated the samples overnight with the protein beads and the anti-C-Myc antibody. Through SDS-page, they were able to resolve equal amounts of proteins. Given the sample, they targeted protein A through antibodies. They then, used ECL to pull down protein A from the cell. They, then, tag the protein with another antibody, this time specific to protein B. If protein B is tagged to protein A then the antibody will bind, and if it wasn’t there then you wouldn’t see any binding. In the following experiment, similar anti-body tags will be used for the proteins: DISC1 and NDEL1. 

* 1. **Co-localization**

Colocalization is a common method used to observe the spatial overlap between different fluorescent labels to see if the different targets are located in the same area of the cell - or at least close to each other. This method was employed by Firbourg et al. (2011) to make sure mGluR2 was expressed in same location as 2AR. Taking a sample of the proteins, incubating them overnight, and giving them fluorescent labels are the first steps needed for this method. Then, using fluorescence microscopy, a picture of the cells are taken to compare the different emission wavelength of the different fluorescent labels used. If there is an overlap, then the targets are either in the same location of the cell, or very near to one another. The final image will look similar to Figure 2, depending on results. 

Knockout of the NDEL1 protein will then occur to effectively test the influence of this protein on the function of DISC1.

* 1. **Confocal Imaging**

Estimation of spine formation will occur through confocal imaging. This method was employed by Golden et al. (2013) to acquire images of a spine analysis. Images were acquired on a confocal LSM 710 for morphological analysis. Neurons were randomly selected from the NAc shell. Dendritic segments were then imaged using a hundred times lens and a zoom of 2.5. A total of ~2,500 dendritic spines were analyzed, with about 2 dendrites per neuron, with 5 neurons per mouse being analyzed. This particular experiment utilized Neuron studio, a program that classifies spines as thin, mushroom, or stubby on the basis of various values, such as diameter, aspect ratio, and head-to-neck ratio.

1. **Discussion**

If all goes well, the confocal images of the spine formation will exhibit clear differences between NDEL1 knockout and no NDEL1 knockout. Similar to the results exhibited from Golden et al. (2013), the confocal images hope to show an increase of stubby and thin dendritic spines (Figure 3). These results may lead me to the conclusion of the importance in NDEL1 in schizophrenia and DISC1 function, ultimately leading me to suggest a possible new drug therapy that targets these two proteins. I would, again, remind the reader of the importance of these results and how they could possibly lead to treatment of schizophrenia-like symptoms. Unfortunately, those perfect results are not guaranteed - and even more so, if they were, much more research would need to be done focusing on finding more information on DISC1 and NDEL1 separately.

Another result that could present is that there is no dendritic spine changes - or, that it is actually more ‘mushroom’ dendritic observances seen in those with the knockout of NDEL1. The first results - of no change or difference - would be disheartening in that it would show this relationship is not important or vital for schizophrenia. However, it will still bring us closer to where we started. The other set of results, that the knockout actually positively impacts spine formation in the hippocampus region would be more so revolutionary since the evidence thus far does not suggest that. Ultimately, though, it would lead to a bigger conversation on the relationship between these two proteins and schizophrenia, which could serve as insight on what is still to come.

Interpreting these results, too will be difficult. A majority of these results are qualitative-based, which makes it much harder to be objective. As you can see from Figure 2, interpreting results from colocalization specifically, can be a little more difficult. It adds room for bias in being able to look at the image and seeing the outcome you want. While they have various systems and programs in place to assist with this, so as to stay objective in science, it can still be a little more difficult to truly know that your results are what you think they are - and that the lighting in the image is not suggesting differently.

Despite these possible problems and results, DISC1 and NDEL1 are important proteins that can help us learn more about schizophrenia and neuroplasticity within the brain. With appropriate attention, they can open doors to new drug therapies that may help those suffering with schizophrenia in bettering their prognosis. These advancements would not only save our economy money, but also give thousands of people the opportunity to live better, more full lives.



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