Amanda Atrash

**Disruption of DISC1 via knockdown of NDEL1 stunts dendritic spine structure 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 not 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.

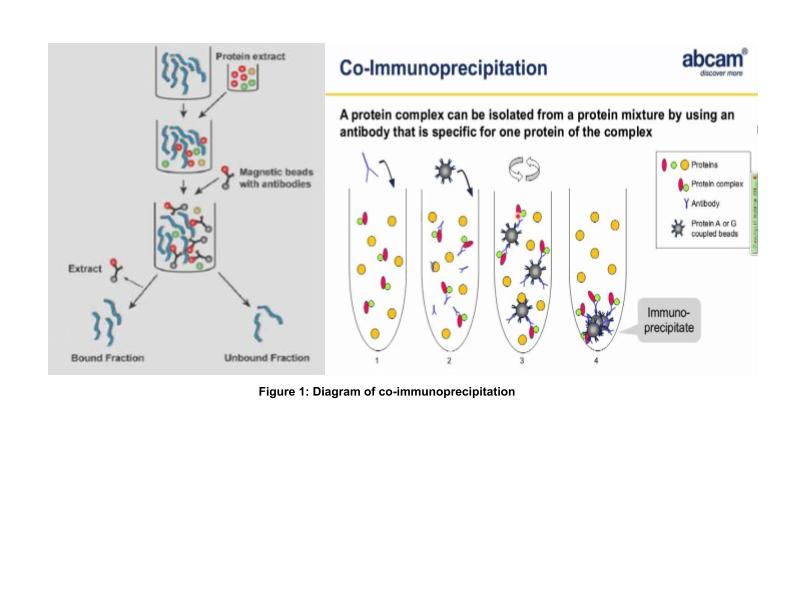
One of DISC1’s many binding partners includes Nuclear Distribution Element-like 1 (NDEL1). NDEL1 is a centrosomal protein that is involved in mitosis, neuronal migration, neuroplasticity, and neurogenesis during brain development (Burdick et al., 2008). Neural plasticity refers to the brain’s ability 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 (Nicodemus et al., 2010, 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 knockdown of one protein affect the function of the other? How does the knockdown 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 knockdown of NDEL1 impacts the function of DISC1 in the spine formation of the hippocampus region.

1. **Experiment**

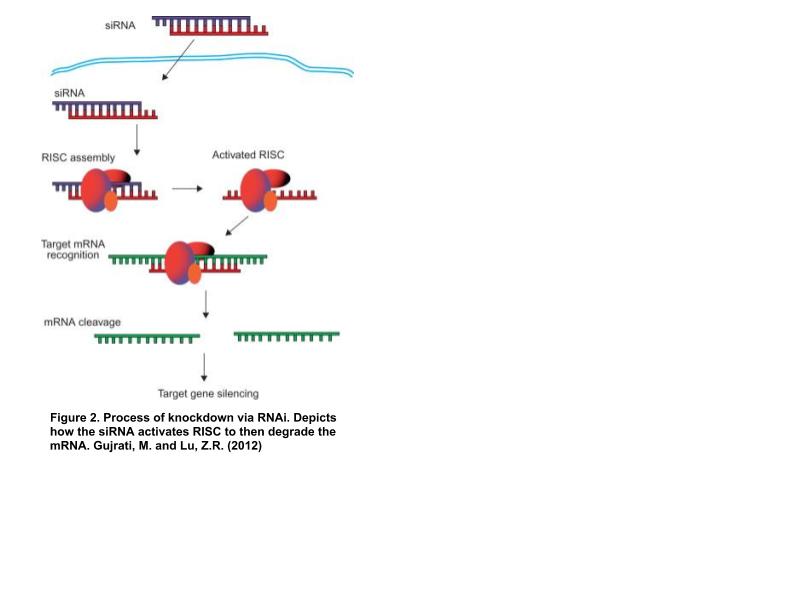
The aim of this experiment is to understand the relationship between the proteins NDEL1 and DISC1 in the development of the spine structure in the hippocampus region of the brain. Samples of the two proteins will be injected inside adult mice to gather and measure the dendritic spine growth and formation within the hippocampus region. In these samples, NDEL1 will be knocked down 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 knockdown, 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 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. In Kayima et al. (2006), the antibodies used against DISC1 were raised in rabbits against amino acids 360-374 of rodent DISC1 and affinity-purified. The antibody for DISC1, then, would be rabbit polyclonal anti-DISC1 antibody and the antibody for NDEL1 is mouse monoclonal antibody, clone OTI1G10. Both of these can be purchased online for the purposes of this experiment. For this experiment, this would first occur in tissue cells, starting with anti-HA and anti-Myc antibodies then done again in the mouse brain with anti-DISC1 and anti-NDEL1 antibodies.

* 1. **Knockdown of NDEL1**

The knockdown of NDEL1 in the NDEL1-DISC1 protein-protein relationship will occur to understand how NDEL1 impacts DISC1 and spine formation. The original plan was to knockout the NDEL1 protein, however, further research has shown that knockout of NDEL1 can lead to embryonic lethality and is not viable for life (Sasaki et al., 2005). This is primarily due to the various roles NDEL1 plays in modulate dynein function, coupling of the centrosome and nucleus during neuronal migration, and in determining neuronal positioning (Sasaki et al., 2005, Toth et al., 2008).

Instead of completely silencing the protein, a knockdown of NDEL1 will occur via RNA interference (RNAi). RNAi uses molecules of RNA that are complementary to the mRNA of the gene of interest. As shown in Figure 2, these molecules bind to mRNA and inhibit its translation into protein through the cytoplasmic breakdown of the double stranded RNA into small interfering RNA (siRNA) (Gujrati, M. and Lu, Z.R., 2012). These siRNA fragments, generally 20–25 nucleotides in length, incorporate into the RNA-induced silencing complex (RISC). RISC unwinds siRNA into single-stranded RNA and can then seek out and target the complementary mRNA (Gujrati, M. and Lu, Z.R., 2012). But not all mRNA molecules will be bound to its complement, meaning some might escape and get translated to give the functional protein, thus not completely eliminating the protein.

Similar to the method used in Hayashi et al. (2010), NDEL1 will be knocked down via RNAi. In their experiment, all the deletion and point-mutated DISC1 and NDEL1 expression constructs were made by PCR-based mutagenesis protocol. Plasmids expressing interfering short hairpin RNA (shRNA) were generated to suppress NDEL1 protein expression. Six shRNA plasmids were produced and tested by the extent of suppression in rat NDEL1 in HEK293 cells (Kaimiya et al., 2006, Hayashi et al., 2010). After suppression was confirmed, the selected shRNAs were tested further in PC12 rat cells. All RNAi constructs were tested by both western blot and immunocytochemical staining. One of the two RNAi sequences used and tested to reduce Ndel1 expression is 5′-GGAGAAACTAGAGCATCAG-3′ (Kamiya et al., 2006, Hayashi et al., 2010). This sequence strongly suppresses the NDEL1 protein compared to the other sequence that is tested and listed, and is to be used solely for rodent NDEL1, not human NDEL1 as there are some slight, yet important, differences in their expression. Various experimenters have used this similar method and construct to complete a knockdown of NDEL1, suggesting high rates of success.

For the purposes of this experiment, the sequence listed above would be used as the RNAi construct to knockdown NDEL1.

* 1. **Confocal Imaging**

Estimation of dendritic spine structure 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. A similar analysis will be done in this experiment.

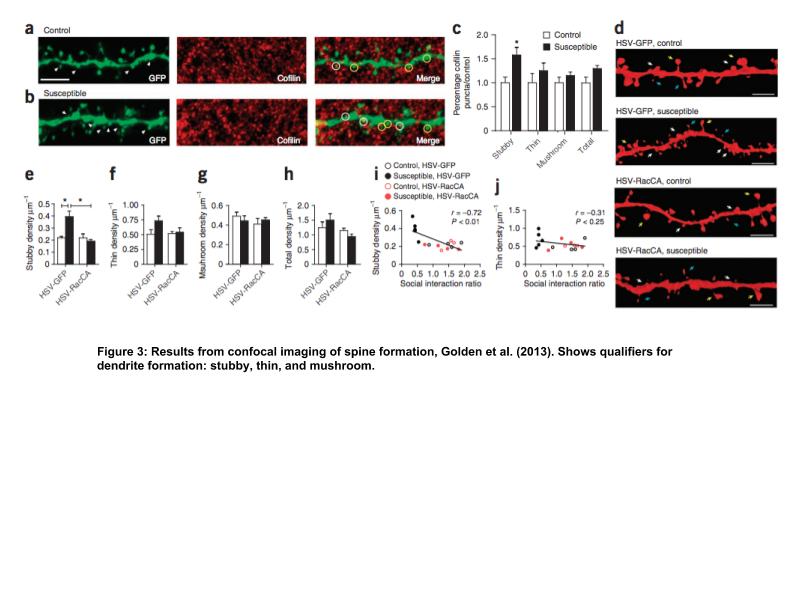
1. **Discussion**

If all goes well, the confocal images of the spine formation will exhibit clear differences between NDEL1 knockdown and no NDEL1 knockdown. 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 knockdown 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 knockdown 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 3, interpreting results from confocal imaging 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.

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.



1. **References**
2. Golden, S. A., Christoffel, D. J., Hodes, G. E., Heshmati, M., Magida, J., Davis, K., … Russo, S. J. (2013). Epigenetic regulation of synaptic remodeling in stress disorders. Nature Medicine, 19(3), 337–344. http://doi.org/10.1038/nm.3090 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3594624/)
3. Fribourg, M., Moreno, J. L., Holloway, T., Provasi, D., Baki, L., Mahajan, R., … Logothetis, D. E. (2011). Decoding the Signaling of a GPCR Heteromeric Complex Reveals a Unifying Mechanism of Action of Antipsychotic Drugs. Cell, 147(5), 1011–1023. http://doi.org/10.1016/j.cell.2011.09.055 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3255795/)
4. Moreno, J. L., Muguruza, C., Umali, A., Mortillo, S., Holloway, T., Pilar-Cuéllar, F., … González-Maeso, J. (2012). Identification of Three Residues Essential for 5-Hydroxytryptamine 2A-Metabotropic Glutamate 2 (5-HT2A·mGlu2) Receptor Heteromerization and Its Psychoactive Behavioral Function. The Journal of Biological Chemistry, 287(53), 44301–44319. http://doi.org/10.1074/jbc.M112.413161 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3531745/)
5. Namba, T., Ming, G.-l., Song, H., Waga, C., Enomoto, A., Kaibuchi, K., Kohsaka, S. and Uchino, S. (2011), NMDA receptor regulates migration of newly generated neurons in the adult hippocampus via Disrupted-In-Schizophrenia 1 (DISC1). Journal of Neurochemistry, 118, 34–44. doi:10.1111/j.1471-4159.2011.07282.x (http://onlinelibrary.wiley.com/doi/10.1111/j.1471-4159.2011.07282.x/full)
6. Soares, D. C., Carlyle, B. C., Bradshaw, N. J., & Porteous, D. J. (2011). DISC1: Structure, Function, and Therapeutic Potential for Major Mental Illness. ACS Chemical Neuroscience, 2(11), 609–632. http://doi.org/10.1021/cn200062k (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3222219/)
7. Katherine E. Burdick, Atsushi Kamiya, Colin A. Hodgkinson, Todd Lencz, Pamela DeRosse, Koko Ishizuka, Sarah Elashvili, Hiroyuki Arai, David Goldman, Akira Sawa, Anil K. Malhotra; Elucidating the relationship between DISC1, NDEL1 and NDE1 and the risk for schizophrenia: Evidence of epistasis and competitive binding . Hum Mol Genet 2008; 17 (16): 2462-2473. doi: 10.1093/hmg/ddn146 (https://academic-oup-com.proxy.library.vcu.edu/hmg/article/17/16/2462/article)
8. Kim, J., Liu, C., Zhang, F., Duan, X., Wen, Z., Song, J., Feighery, E., Lu, B., Rujescu, D., St Clair, D., Christian, K., Callicott, J., Weinberger, D., Song, H., and Ming, G. (2012), Interplay between DISC1 and GABA Signaling Regulates Neurogenesis in Mice and Risk for Schizophrenia. Cell Press, 148(5), 1051 - 1064. doi : http://dx.doi.org/10.1016/j.cell.2011.12.037 (http://www.cell.com/cell/abstract/S0092-8674(12)00159-6?\_returnURL=http%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867412001596%3Fshowall%3Dtrue)
9. Ellaithy, A., Younkin, J., Gonzalez-Maeso, J., & Logothetis, D. E. (2015). Positive Allosteric Modulators of Metabotropic Glutamate 2 Receptors in Schizophrenia Treatment. Trends in Neurosciences, 38(8), 506–516. http://doi.org/10.1016/j.tins.2015.06.002 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4530036/)
10. Moreno, J. L., Kurita, M., Holloway, T., López, J., Cadagan, R., Martínez-Sobrido, L., … González-Maeso, J. (2011). Maternal influenza viral infection causes schizophrenia-like alterations of 5-HT2A and mGlu2 receptors in the adult offspring. The Journal of Neuroscience : The Official Journal of the Society for Neuroscience, 31(5), 1863–1872. http://doi.org/10.1523/JNEUROSCI.4230-10.2011 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3037097/)
11. Voineskos, D., Rogasch, N.C., Rajji, T. K., Daskalakis, Z. (2013). A Review of Evidence Linking Disrupted Neural Plasticity to Schizophrenia. Canadian journal of psychiatry. PubMed, 58(2), 86 - 92. doi : 10.1177/070674371305800205 (https://www.researchgate.net/publication/235739948\_A\_Review\_of\_Evidence\_Linking\_Disrupted\_Neural\_Plasticity\_to\_Schizophrenia)
12. Keshavan, M., Mehta, U., Padmanabhan, J., & Shah, J. (2015). Dysplasticity, metaplasticity, and schizophrenia: Implications for risk, illness, and novel interventions. Development and Psychopathology, 27(2), 615-635. doi:10.1017/S095457941500019X
13. González-Maeso, J., Ang, R., Yuen, T., Chan, P., Weisstaub, N. V., López-Giménez, J. F., … Sealfon, S. C. (2008). Identification of a Novel Serotonin/Glutamate Receptor Complex Implicated in Psychosis. Nature, 452(7183), 93–97. http://doi.org/10.1038/nature06612 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2743172/)
14. Duan, X., Chang, J., Faulkner, R., Kim, J., Kitabatake, Y., Liu, X., Yang, C., Jordan, J., Ma, D., Liu, C., Ganesan, S., Cheng, H., Ming, G., and Song, H.(2007), Disrupted-In-Schizophrenia 1 Regulates Integration of Newly Generated Neurons in the Adult Brain. Cell Press, 130(6):,1146 - 1158. doi: http://dx.doi.org/10.1016/j.cell.2007.07.010 (http://www.sciencedirect.com/science/article/pii/S0092867407008975)
15. Sasaki, S., Mori, D., Toyo-oka, K., Chen, A., Garrett-Beal, L., Muramatsu, M., … Hirotsune, S. (2005). Complete Loss of Ndel1 Results in Neuronal Migration Defects and Early Embryonic Lethality. Molecular and Cellular Biology, 25(17), 7812–7827. http://doi.org/10.1128/MCB.25.17.7812-7827.2005 (https://www-ncbi-nlm-nih-gov.proxy.library.vcu.edu/pmc/articles/PMC1190282/)
16. Shinoda, T., Taya, S., Tsuboi, D., Hikita, T., Matsuzawa, R., Kuroda, S., Iwamatsu, A., and Kaibuchi, K. (2007), DISC1 Regulates Neurotrophin-Induced Axon Elongation via Interaction with Grb2. Journal of Neuroscience, 27(1), 4 - 14. doi: https://doi.org/10.1523/JNEUROSCI.3825-06.2007 (http://www.jneurosci.org/content/27/1/4)
17. Kim, J. Y., Duan, X., Liu, C. Y., Jang, M., Guo, J. U., Pow-anpongkul, N., . . . Ming, G. (2009). DISC1 regulates new neuron development in the adult brain via modulation of AKT-mTOR signaling through KIAA1212. Neuron, 63(6), 761-73. doi:http://dx.doi.org.proxy.library.vcu.edu/10.1016/j.neuron.2009.08.008 (http://www.sciencedirect.com/science/article/pii/S0896627309006175)
18. Olivia Engmann, Tibor Hortobágyi, Ruth Pidsley, Claire Troakes, Hans-Gert Bernstein, Michael R. Kreutz, Jonathan Mill, Margareta Nikolic, Karl Peter Giese; Schizophrenia is associated with dysregulation of a Cdk5 activator that regulates synaptic protein expression and cognition. Brain 2011; 134 (8): 2408-2421. doi: 10.1093/brain/awr155 (https://academic.oup.com/brain/article/134/8/2408/356279/Schizophrenia-is-associated-with-dysregulation-of)
19. Nestler, E., Peña, C., Kundakovic, M., Mitchell, A., and Akbarian, S. (2016). Epigenetic Basis of Mental Illness. The Neuroscientist, 22(5), 447 - 463. doi: 10.1177/1073858415608147 (http://journals.sagepub.com/doi/abs/10.1177/1073858415608147)
20. Kurita, M., Holloway, T., García-Bea, A., Kozlenkov, A., Friedman, A. K., Moreno, J. L., & ... Buxbaum, J. D. (2012). HDAC2 regulates atypical antipsychotic responses through the modulation of mGlu2 promoter activity. Nature Neuroscience, 15(9), 1245-1254. doi: 10.1038/nn.3181 (http://www.nature.com/neuro/journal/v15/n9/full/nn.3181.html)
21. Amort T, Rieder D, Wille A, Khokhlova-Cubberley D, Riml C, Trixl L, Jia X-Y, Micura R, LusserEmail A (2017). Distinct 5-methylcytosine profiles in poly(A) RNA from mouse embryonic stem cells and brain. Genome Biol 18:1. (https://genomebiology.biomedcentral.com/articles/10.1186/s13059-016-1139-1)
22. Hanks, J. B., & González-Maeso, J. (2013). Animal Models of Serotonergic Psychedelics. ACS Chemical Neuroscience, 4(1), 33–42. http://doi.org/10.1021/cn300138m (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3547517/)
23. Holloway, T., & González-Maeso, J. (2015). Epigenetic Mechanisms of Serotonin Signaling. ACS Chemical Neuroscience, 6(7), 1099–1109. http://doi.org/10.1021/acschemneuro.5b00033 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4838281/)
24. Akbarian, S. (2014). Epigenetic mechanisms in schizophrenia. Dialogues in Clinical Neuroscience, 16(3), 405–417. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4214181/)
25. Bradshaw, N.J. & Hayashi, M.A.F. Cell. Mol. Life Sci. (2017) 74: 1191. doi:10.1007/s00018-016-2395-7(https://link-springer-com/article/10.1007/s00018-016-2395-7)
26. Toth C, Shim SY, Wang J, Jiang Y, Neumayer G, Belzil C, et al. (2008) Ndel1 Promotes Axon Regeneration via Intermediate Filaments. PLoS ONE 3(4): e2014. https://doi.org/10.1371/journal.pone.0002014 (http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0002014#s4)
27. Nicodemus, K.K., Callicott, J.H., Higier, R.G. et al. Hum Genet (2010) 127: 441. doi:10.1007/s00439-009-0782-y (https://link.springer.com/article/10.1007/s00439-009-0782-y)
28. Kamiya, A., Kubo, K., Tomoda, T., Takaki, M., Youn, R., Ozeki, Y., & ... Sawa, A. (2005). A schizophrenia-associated mutation of DISC1 perturbs cerebral cortex development. Nature Cell Biology, 7(12), 1067-1082. doi:10.1038/ncb1328 (http://web.b.ebscohost.com/ehost/detail/detail?sid=824fa02e-24a6-4769-aded-d4d091759dd3%40sessionmgr103&vid=0&hid=102&bdata=JkF1dGhUeXBlPWlwLHVybCxjb29raWUsdWlkJnNpdGU9ZWhvc3QtbGl2ZSZzY29wZT1zaXRl#AN=19004252&db=a9h)
29. Atsushi Kamiya, Toshifumi Tomoda, Jennifer Chang, Manabu Takaki, Caixin Zhan, Masahiko Morita, Matthew B. Cascio, Sarah Elashvili, Hiroyuki Koizumi, Yasukazu Takanezawa, Faith Dickerson, Robert Yolken, Hiroyuki Arai, Akira Sawa; DISC1–NDEL1/NUDEL protein interaction, an essential component for neurite outgrowth, is modulated by genetic variations of DISC1. Hum Mol Genet 2006; 15 (22): 3313-3323. doi: 10.1093/hmg/ddl407 (https://academic-oup-com.proxy.library.vcu.edu/hmg/article-lookup/doi/10.1093/hmg/ddl407)
30. Gujrati, M. and Lu, Z.R. (2012). Targeted Systemic Delivery of Therapeutic siRNA. Jeffrey Schlom, *Gene Therapy of Cancer* (pp. 47 - 65). Oxford, UK: Elsevier Inc.