Proceedings of the 2nd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/46/20240605

Can BDNF manipulate the overshoot-and-decline effect of adult neurogenesis during recovery from sleep deprivation?

Zhi Feng Etan Kiang

Harvard-Westlake School, Studio City, CA91604, USA

tywell45@gmail.com

Abstract. Sleep deprivation reduces an organism's ability to perform adult neurogenesis, the generation of new neurons in adulthood. Recently, researchers discovered that recovery in sleep deprivation causes the rate of adult neurogenesis to overshoot the normal rate, and then decline back to normal. This research focuses on brain-derived neurotrophic factor, and whether or not manipulating it and its related pathways could extend the overshoot effect to enhance adult neurogenesis in the long term. The conclusion that is expected from this research is that BDNF will be able to prolong the overshoot of adult neurogenesis that is induced by sleep deprivation. The work seeks to provide more control over the overshoot-and-decline effect of adult neurogenesis during recovery from sleep deprivation, opening new pathways to treatments involving the manipulation of adult neurogenesis.

Keywords: Brain-derived neurotrophic factor (BDNF), tyrosine receptor kinase B (TrkB), BDNF-TrkB pathway, Bcl2, adult neurogenesis, sleep deprivation

1. Background

I would be very happy to be able to research about brain-derived neurotrophic factor (BDNF) and its possible effect on the overshoot-and-decline effect of adult neurogenesis during recovery from sleep deprivation, as discovered by Mirescu et al. Sleep deprivation affects many people around the world. I want to research in order to understand not only the direct effects of sleep deprivation on adult neurogenesis but also how we can harness the effects to help those who struggle with neurological ailments. My research aims to find ways to manipulate the adult neurogenesis overshoot, and I believe that our findings can suggest possible treatments for decreased adult neurogenesis in seniors or victims of neurodegenerative diseases.

In a 2006 PNAS paper published by Mirescu et al., the researchers found that while sleep deprivation did result in decreased adult neurogenesis through the upregulation of glucocorticoids, adult neurogenesis levels exhibited a rebound during the recovery period unrelated to glucocorticoid levels before returning to control levels [1]. Glucocorticoids were already known to have a negative impact on adult neurogenesis by suppressing proliferation of radial stem cells and reducing neuroblast numbers [2]. Sleep deprivation increases stress, which is a manifestation of increased glucocorticoid levels [3]. The BDNF-TrkB pathway also plays a vital role in adult neurogenesis; not only does BDNF promote cell proliferation, but its downstream product Bcl2 also discourages apoptosis [4].

DOI: 10.54254/2753-8818/46/20240605

2. Literature review

Existing research shows that the rebound in adult neurogenesis after one week of recovery from sleep deprivation is not affected by glucocorticoid levels, and that adult neurogenesis levels decrease to normal after three weeks [1]. I find the rebound effect particularly compelling to the discussion of how adult neurogenesis can be enhanced to normal levels in patients with neurodegenerative diseases. The fact that the adult neurogenesis overshoot-and-decline effect does not rely on glucocorticoid levels being lowered as Mirescu and company first imagined could lead to a more consistent treatment method.

I also read that sleep deprivation decreases BDNF levels, which made me curious about the impact that manipulating BDNF levels could have on sleep deprivation [5]. I asked myself if I would be able to dampen or even reverse the negative effects of sleep deprivation on adult neurogenesis by promoting BDNF. My interest in sleep deprivation was immediately piqued by the literature I read.

The BDNF-TrkB pathway is a MAP kinase pathway that not only promotes cell proliferation but also discourages cell apoptosis through the promotion of Bcl2 [6]. BDNF binds to TrkB receptors, which are tyrosine kinase receptors. This activates Erk5 and promotes the downstream production of Bcl2, which encourages senescent cell survival [7]. Bcl2's role in cell survival means that it could be used to sustain the overshoot effect in recovery from sleep deprivation by discouraging apoptosis of new neurons.

Tracking new neurons in a rat's brain is usually done by injecting BrdU into the brain of a mouse, and then using an inverted fluorescence microscope after the rat has been perfused and its brain sectioned. Stem cells, which are indicators of new neurons forming, cycle slowly and retain their BrdU labels well, reducing the need to constantly reinject BrdU [8].

3. Research approach

I predict that my experiment will show that BDNF and Bcl2 can manipulate the overshoot-and-decline effect of adult neurogenesis. My experiment will involve the detection of new neurons through inverted fluorescence microscopy and BrdU indicator. However, BrdU has toxic effects on cells, so groups expected to yield a normal level of new neurons may yield slightly less [9].

In Mirescu's experiment, he sleep-deprived rats for 72 hours, injected their brains with BrdU indicator, then allowed them to recover for 2 hours, 1 week, or 3 weeks before perfusing them and counting new neurons with the BrdU [1].

My first experiment will involve four groups of Sprague-Dawley rats; cage control (CC), small-platform sleep deprivation, cage control with TrkB inhibited during the initial 72-hour period, and small-platform sleep deprivation with TrkB inhibited during the recovery period. All groups will have sub-groups divided into 2-hour recovery to check adult neurogenesis levels right after sleep deprivation, as well as 1-week and 3-week recovery. This will allow me to check if the overshoot phase of sleep deprivation recovery is caused by BDNF. Small-platform sleep deprivation prevents REM sleep in rats by ensuring that they will fall into a pool of water and be forced to climb back up if they fall into REM sleep [10]. TrkB inhibition will be achieved by carefully administering Larotrectinib, a breakthrough cancer treatment that inhibits tyrosine receptor kinases [11]. The main advantage to my experiment method is how definitive the results can be; if cage control with TrkB inhibited during the first 72 hours has no effect versus cage control, I will know that BDNF does not play a role in the overshoot-and-decline effect. If small-platform sleep deprivation with TrkB inhibited during the recovery period does nothing, I will also know that BDNF does not play a role in the overshoot-and-decline effect. A drawback is that I will have to control the dosage of larotrectinib very carefully in the group that receives TrkB inhibition in the initial 72-hour period to make sure that the effects of the larotrectinib do not bleed into the recovery period.

My second experiment focuses on Bcl2 and its possible role in affecting the overshoot pattern by reducing apoptosis of new neurons. It will involve three groups of rats; cage control, small platform, and small platform with Bcl2 promoted during the recovery period. All groups will have sub-groups divided into 2-hour recovery to check adult neurogenesis levels right after sleep deprivation, as well as 1-week and 3-week recovery. Simply promoting Bcl2 through increasing BDNF levels will also induce

DOI: 10.54254/2753-8818/46/20240605

cell proliferation, inflating cell count without knowing the true effect of the reduced apoptosis alone [12]. Promoting Bcl2 alone can be done through acridone derivative A22 [13]. Finding the effect of Bcl2 on the decline phase of sleep deprivation will allow me to learn how to extend the overshoot in adult neurogenesis. The main advantage is that the negative result of the experiment is already known; if promoting Bcl2 has no effect, the level of new neurons will simply return to control levels as shown in Mirescu's experiment [1].

4. Methodology

The first step is to collect at least 105 Sprague-Dawley rats. Every sub-group will require 5 rats averaged for maximum accuracy, and there are 7 total experimental groups each containing 3 sub-groups for the different recovery times. The small-platform sleep deprivation test is inexpensive and very feasible; it requires an upside-down flowerpot for the mouse to stand on inside a bucket of water.

4.1. Animal Treatment and Ethical Methods

The experiment shall be performed to cause the least amount of pain to the rats. BrdU will be injected into the rats' brains, and after a recovery period of 2 hours, 1 week, or 3 weeks, the rats will be perfused with 4.0% paraformaldehyde in 0.1M phosphate buffer to ensure that they die without pain [1]. Their dentate gyruses will be sectioned into 40 micrometer thick slices for surveying with an inverted fluorescence microscope [14].

4.2. Inverted Fluorescence Microscopy

After using BrdU to mark the new neurons in the rat hippocampus, the slides of dentate gyrus will be processed just as they were in Mirescu's sleep deprivation experiment [1]. Then, I will count the BrdU-labeled cells at 1000x zoom using a light microscope and multiply the count by 24 to estimate the count of BrdU-labeled cells per brain.

4.3. Interpretation of Results

The number of BrdU-labeled cells will indicate the number of new neurons during the recovery time after sleep deprivation. A decreased count of BrdU-labeled cells for the 1-week recovery subgroup when the BDNF-TrkB pathway is inhibited would indicate that BDNF has a significant impact on the overshoot effect of recovery from sleep deprivation, because without it the overshoot effect would not happen. An increased count of BrdU-labeled cells for the 3-week recovery subgroup when Bcl2 is promoted would indicate that Bcl2 plays a vital role in the regulation of apoptosis of new neurons in the decline stage of sleep deprivation recovery.

Bar graphs with each bar corresponding to each recovery time will be used to intuitively visualize the change in BrdU-labeled new neuron count. As depicted in Mirescu's experiment, the BrdU-labeled cell count was lowest after 2 hours of recovery, spiked during 1 week of recovery, and then returned to normal after 3 weeks of recovery. Any deviation from this control will suggest that BDNF and Bcl2 have a significant impact on the overshoot-and-decline effect of recovery from sleep deprivation.

DOI: 10.54254/2753-8818/46/20240605

5. Expected results

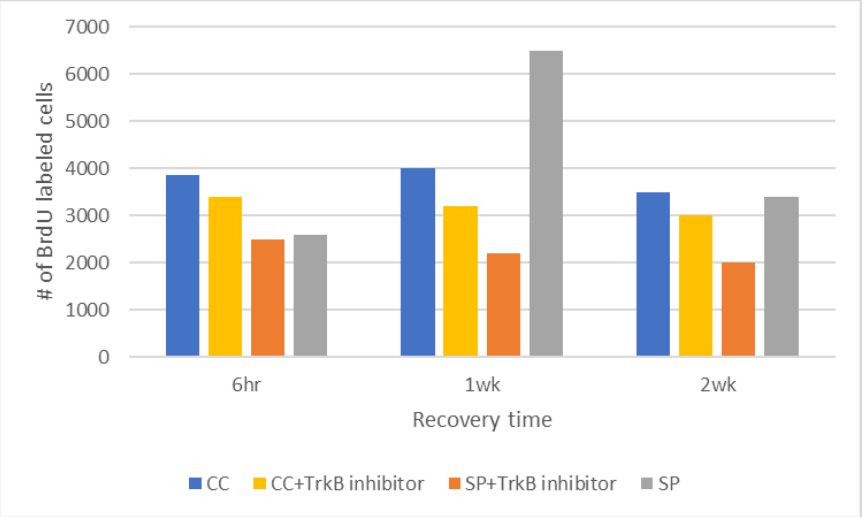

Figure 1. Expected raw data from the first experiment [15]

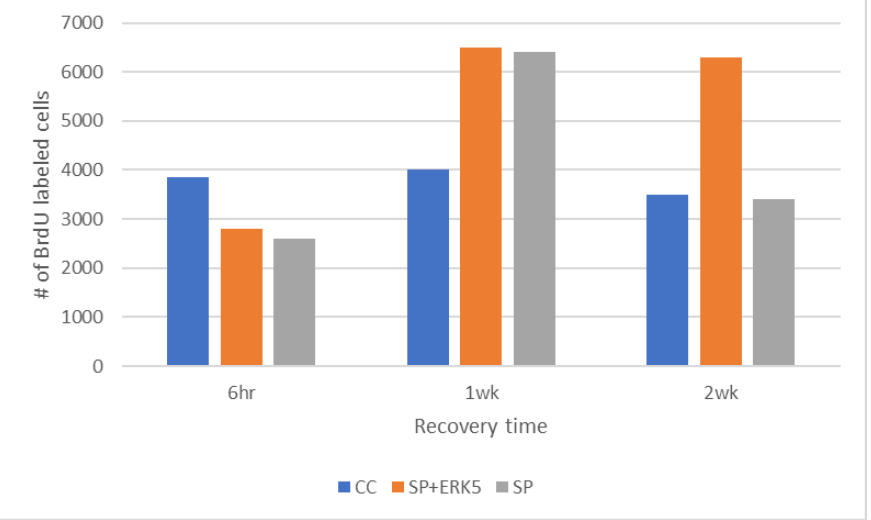

Figure 2. Expected raw data from the second experiment [15]

I expect that BDNF and Bcl2 will affect the overshoot-and-decline effect of adult neurogenesis in sleep deprived rats. BDNF is known to induce growth for neurons, as well as cancer cells and a wide range of other cells [12]. Bcl2 is a main factor in regulation of apoptosis, which most likely drives the decline process of new neurons. Even if the decline in new neurons is not totally mitigated due to other factors in apoptosis, the addition of Bcl2 will at least slow the decline of new neuron count. The possibilities that my research can lead to are vast; BDNF is already being investigated as a therapeutic agent in Parkinson's disease. The notion that sleep deprivation could induce BDNF as shown in the overshoot phase could lead to potential treatment that does not require external stimulation of BDNF, especially since a 2020 experiment by Palasz et al showed that external dosage of BDNF did not relieve symptoms of Parkinson's disease [16]. Parkinson's disease and other neurodegenerative diseases are often long-term and require sustained treatment, so a short burst in BDNF will not be adequate. Assuming that my hypothesis is correct, Bcl2 will be vital in maintaining elevated levels of new neurons.

BDNF has also been found by Björkholm and Monteggia to be an important bridge between antidepressant drugs and the resulting neuroplastic changes that alleviate symptoms of depression [17].

DOI: 10.54254/2753-8818/46/20240605

A drug like acridone derivative A22 after recovery from sleep deprivation could provide a sustained alternative treatment for depression.

6. Conclusion

I genuinely believe that my proposal to research about BDNF and its possible effect on the overshoot-and-decline effect of adult neurogenesis during recovery from sleep deprivation can bring about a novel understanding of sleep deprivation's role in neurological ailment. Being able to manipulate BDNF would allow us to at least slow the symptoms of a neurological ailment, since BDNF is known to be important to at least Parkinson's disease and depression. The ability to control Bcl2 levels to allow for sustained treatment would help us develop a drug that could provide long-term aid to those who suffer from neurological ailments. For the depressed and for the neurologically diseased, I urge you to consider my research for funding.

References

[1] Mirescu, C., Peters, J. D., Noiman, L., & Gould, E. (2006). Sleep deprivation inhibits adult neurogenesis in the hippocampus by elevating glucocorticoids. *Proceedings of the National Academy of Sciences*, *103*(50), 19170–19175. https://doi.org/10.1073/pnas.0608644103

[2] Cope, E. C., & Gould, E. (2019). Adult neurogenesis, glia, and the extracellular matrix. *Cell Stem Cell*, *24*(5), 690–705. https://doi.org/10.1016/j.stem.2019.03.023

[3] Nollet, M., Wisden, W., & Franks, N. P. (2020). Sleep deprivation and stress: A reciprocal relationship. *Interface Focus*, *10*(3), 20190092. https://doi.org/10.1098/rsfs.2019.0092

[4] Warren, C. F. A., Wong-Brown, M. W., & Bowden, N. A. (2019). BCL-2 family isoforms in apoptosis and cancer. *Cell Death & Disease*, *10*(3), 177. https://doi.org/10.1038/s41419-019-1407-6

[5] Wadhwa, M., Prabhakar, A., Ray, K., Roy, K., Kumari, P., Jha, P. K., Kishore, K., Kumar, S., & Panjwani, U. (2017). Inhibiting the microglia activation improves the spatial memory and adult neurogenesis in rat hippocampus during 48 h of sleep deprivation. *Journal of Neuroinflammation*, *14*(1), 222. https://doi.org/10.1186/s12974-017-0998-z

[6] Anerillas, C., Herman, A. B., Munk, R., Garrido, A., Lam, K.-W. G., Payea, M. J., Rossi, M., Tsitsipatis, D., Martindale, J. L., Piao, Y., Mazan-Mamczarz, K., Fan, J., Cui, C.-Y., De, S., Abdelmohsen, K., de Cabo, R., & Gorospe, M. (2022). A BDNF-TrkB autocrine loop enhances senescent cell viability. *Nature Communications*, *13*(1), 6228. https://doi.org/10.1038/s41467-022-33709-8

[7] Morrison, D. K. (2012). MAP kinase pathways. *Cold Spring Harbor Perspectives in Biology*, *4*(11), a011254. https://doi.org/10.1101/cshperspect.a011254

[8] Zhang, L., Li, H., Zeng, S., Chen, L., Fang, Z., & Huang, Q. (2015). Long-term tracing of the BrdU label-retaining cells in adult rat brain. *Neuroscience Letters*, *591*, 30–34. https://doi.org/10.1016/j.neulet.2015.02.023

[9] Taupin, P. (2007). BrdU immunohistochemistry for studying adult neurogenesis: Paradigms, pitfalls, limitations, and validation. *Brain Research Reviews*, *53*(1), 198–214. https://doi.org/10.1016/j.brainresrev.2006.08.002

[10] Kitka, T., Katai, Z., Pap, D., Molnar, E., Adori, C., & Bagdy, G. (2009). Small platform sleep de privation selectively increases the average duration of rapid eye movement sleep episodes du ring sleep rebound. *Behavioural Brain Research*, *205*(2), 482–487. https://doi.org/10.1016/j. bbr.2009.08.004

[11] Mota-George, G., & Schneider, S. M. (2021). Larotrectinib: A Targeted Therapy for Solid Tumo rs. *Clinical Journal of Oncology Nursing*, *25*(2), 181–187. https://doi.org/10.1188/21.CJON. 181-187

[12] Malekan, M., Nezamabadi, S. S., Samami, E., Mohebalizadeh, M., Saghazadeh, A., & Rezaei, N. (2023). BDNF and its signaling in cancer. *Journal of Cancer Research and Clinical Oncology*, *149*(6), 2621–2636. https://doi.org/10.1007/s00432-022-04365-8

[13] Li, X., Wang, J., Gong, X., Zhang, M., Kang, S., Shu, B., Wei, Z., Huang, Z.-S., & Li, D. (2020). Upregulation of BCL-2 by acridone derivative through gene promoter i-motif for alleviating liver damage of NAFLD/NASH. *Nucleic Acids Research*, *48*(15), 8255–8268. https://doi.org/10.1093/nar/gkaa615

[14] Lein, P. J., Barnhart, C. D., & Pessah, I. N. (2011). Acute Hippocampal Slice Preparation and Hippocampal Slice Cultures. *Methods in Molecular Biology (Clifton, N.J.)*, *758*, 115–134. https://doi.org/10.1007/978-1-61779-170-3_8

[15] Figures made by Zhi Feng Etan Kiang, Zhengyi Sun, and Alvin Zhou, with permission granted by all contributors.

[16] Palasz, E., Wysocka, A., Gasiorowska, A., Chalimoniuk, M., Niewiadomski, W., & Niewiadom ska, G. (2020). BDNF as a Promising Therapeutic Agent in Parkinson's Disease. *Internation al Journal of Molecular Sciences*, *21*(3), 1170. https://doi.org/10.3390/ijms21031170

[17] Björkholm, C., & Monteggia, L. M. (2016). BDNF - a key transducer of antidepressant effects. *Neuropharmacology*, *102*, 72–79. https://doi.org/10.1016/j.neuropharm.2015.10.034