*note: This is the biology research paper that I originally submitted for my English 299 class. Though this is ***NOT MY RESEARCH PROJECT*** for submission to the Library Research Award, I was told it might be of benefit to also provide this paper. My research project infographic, though only describing a fraction of the biological pathway I researched, is derived from a section of this paper, and the evidence exploring this biological pathway in its entirety is also presented here.

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Library Essay: Local Protein Synthesis in Neuronal Dendrites and Its Role in Synaptic Plasticity and Memory

After writing my proposal, I began exploring my initial research question: What impact does local protein synthesis in neuronal dendrites have on the neuron, and how does this inform our understanding of synaptic plasticity and memory? I decided to consider the two clauses of this research question independently, assuming that the second clause builds off the first. Thus, my first set of research aimed simply to understand the function of local protein synthesis in the dendrites - branching nerve cell structures responsible for receiving signals from neighboring neurons("Overview of Neuron Structure and Function (Article)" n.d.)- which led me to a broad review article titled "Dendritic Protein Synthesis, Synaptic Plasticity, and Memory". In an overarching sense, the last 60 years of experimental evidence suggest that local protein synthesis may allow for adaptive adjustments and modifications of the synapse(Sutton and Schuman 2006), which is the small pocket of space between the end terminus of one neuron and the dendrite of a second neuron, responsible for facilitating communication between those cells("Overview of Neuron Structure and Function (Article)" n.d.). Thus, the collective body of scientific work argues that local protein synthesis in dendrites – due to its ability to regulate modifications of the synapse – is critical in the formation and storage of long-term memories(Sutton and Schuman 2006).

However, what struck me about this article was that nearly half of the text was dedicated to unresolved issues, such as the logic underlying what proteins are synthesized in dendrites, questions about the degree of specificity required for synaptic changes, etc. Such a large volume of unanswered queries caused doubt about whether or not there was enough cohesive research to sustain a full examination of my research question; I decided to redirect my research focus towards a broader examination of many sources to see if these sentiments of uncertainty were shared by other researchers. As it turns out, many of the published papers in this field

acknowledge significant gaps in understanding. As summarized by researchers Tang and Schuman:

> Although an increasing number of . . . species [indicative of protein synthesis] have been detected in the dendrite, information about the synaptic synthesis of specific proteins in a physiological context is still limited. The physiological function of synaptic synthesis of specific proteins in . . . neural plasticity expression remains to be shown (Tang and Schuman 2002).

Under the guidance of my instructor and the University of Washington's Biology Subject Librarian, I conducted research to decide the fate of my initial research question – can I find enough concrete, specific evidence to explore it properly and meaningfully? I found such evidence in the BNDF protein, which acts as an "important regulator of synaptic transmission and long-term potentiation . . . in the hippocampus . . . playing a role in the formation of certain forms of memory"(Duarte 2021). BNDF does this by differentially regulating the dendritic localization of mRNA transcripts that code for initiation factors, elongation factor, and aminoacyl-tRNA synthases, all of which are important for translation(Duarte 2021) – the process by which instructions in mRNA are translated into functional proteins(Freeman et al. 2017). Thus, BNDF localizes the molecular machinery necessary to synthesize proteins in the dendrites, which demonstrates how (1) BNDF regulates local protein synthesis in neuronal dendrites and (2) BNDF consequentially regulates the proteins which are made via this local synthesis, and thus their eventual effects on synaptic plasticity. This second conclusion was further supported by the discovery that among the dendritic mRNAs regulated by BNDF, several encode "proteins with synaptic roles such as ionotropic and metabotropic receptors, scaffolding proteins, adhesion molecules, [and] signaling molecules"(Cajigas et al. 2012).

Unearthing such detailed evidence gave me the confidence that my research question was in fact feasible, yet I could not ignore the multitude of questions and unresolved issues I

discovered while researching, especially when they are emphasized so strongly and by so many researchers in this field. Consequentially, I decided to alter my research question. My initial research question had two parts - (1) the effect of local protein synthesis on a single nerve cell and (2) its role in synaptic plasticity and memory – which would be condensed, allowing me the space to also explore unresolved issues. Thus, my final research question became: What role does local protein synthesis in neuronal dendrites play in synaptic plasticity and memory, and what questions remain unanswered regarding this biological mechanism?

At this point, my own research oddly resembled the research I was exploring – a mix of isolated evidence and missing pieces. Through my own research thus far, I had unearthed some specific biological interactions as well as some unanswered questions, so where were the gaps in my knowledge? The BNDF protein demonstrated how local protein synthesis was regulated and that some of these proteins were found to have specific roles in the synapse. But I still did not understand how changes in these synaptic proteins altered synapse plasticity, nor how changes in synaptic plasticity impact memory. These were questions I sought to investigate next, whose answers would hopefully unify the fragmented pieces of evidence I had accumulated thus far.

A theory for how synaptic proteins control synaptic plasticity emerged in the 1940s from neuroscientists Donald Hebb, who hypothesized that “neurons strengthen their communication if the presynaptic cell persistently stimulates the post-synaptic cell. . . Applied to multiple synapses around a group of neurons, [this hypothesis] gave rise to the concept that memories are encoded as . . . biophysical changes to a neuronal network” (Henley and Wilkinson 2013). These biophysical changes can be expressed as “changes in the molecular composition of the postsynaptic membrane”(Henley and Wilkinson 2013) - aka, the dendrites. Since cell membranes are composed primarily of lipids and proteins(Freeman et al. 2017), the connection between

proteins, synaptic plasticity, and memory became clearer: the proteins available in the dendrite determine the protein composition of its postsynaptic membrane, and different postsynaptic membrane compositions – which make the synapse malleable – serve as the mechanism for memory encoding. This made me wonder what localized proteins, if any, have been discovered to play a key role in mediating synaptic plasticity. This same article by Henley and Wilkinson also provided evidence for this question: the most widely researched forms of synaptic plasticity are "induced by activation of postsynaptic . . . NMDA receptors (NMDARs) and expressed by changes in the number of postsynaptic AMPA receptors (AMPARs)", meaning that AMPARs are "critically important for nearly all aspects of brain function, including . . . memory"(Henley and Wilkinson 2013). However, synaptic AMPARs – predominantly made of GluA1 and GluA2 subunits – are assembled in the endoplasmic reticulum(Henley and Wilkinson 2013), which means that they are synthesized via regular protein synthesis in the cell soma, not local protein synthesis in the dendrites. Though not particularly helpful for my research question, this observation seemed logical; if AMPARs are so crucial to synaptic plasticity, synthesizing them through a more centralized system is more efficient and less error-prone than having each dendrite independently synthesize its required AMPARs. Still, such logic made me wonder if there are molecules, perhaps with less vital roles in synaptic plasticity, that are synthesized locally.

Youtes et al. provided a case study of one such molecule. The researchers examined eCBs, which are lipids produced and mobilized by dendrites that travel back across the synapse to the presynaptic cell and suppress the release of neurotransmitter GABA(Younts et al. 2016). GABA is an inhibitory neurotransmitter, meaning that it prevents the nerve cell it binds to from sending a signal(Jewett and Sharma 2020); if Hebb's hypothesis that biophysical changes to

multiple synapses is required for memory encoding holds true, it becomes clear how GABA – which can halt a nerve signal before it catalyzes biophysical changes in those multi-synapse networks – may be an important regulator of synaptic plasticity. Thus, through regulating GABA, eCBs may themselves manage synaptic plasticity.

The researchers found that local dendritic "synthesis [which produces eCB] is required for long-term, but not short-term plasticity of GABA release", and that this synthesis is required for the induction, but not maintenance, of this long-term inhibitory transmission(Younts et al. 2016). Thus, there exists a scientifically examined, locally synthesized molecule – eCB - that impacts synaptic plasticity, but only conditionally. Nevertheless, the conditional impact of this locally synthesized molecule is significant, which prompted me to look for evidence of one more type of molecule to bring this investigation full circle: a protein that is locally synthesized and has consistent, long-term impacts on synaptic plasticity.

The final study I found refocused on NMDAR proteins, which are connected to the previously discussed AMPAR proteins (remember that AMPARs, thought deemed critical for synaptic plasticity, are not synthesized locally). The researchers of this study recognized NMDARs as playing a key role in synaptic plasticity, while also observing that "NMDARs appear to be clustered at synapses, where their realm of influence may be restricted to a small area of post-synaptic membrane, and in the case of dendritic spines, to single postsynaptic sites"(Benson 1997). Considering these two observations, the researchers then hypothesized and tested the idea that "a possible mechanism for the precise targeting or rapid production of NMDARs would be to synthesize them on site". The conclusion was reached that "mRNA encoding NR1, the obligatory subunit of NMDAR, is transported into the dendrites at all stages of dendritic development in cultured hippocampal neurons"(Benson 1997). Therefore, the NR1

protein subunit must be synthesized in the dendrites. The NMDAR protein – made with this subunit - is thus partially synthesized in the dendrites, and most likely assembled in the dendrites, providing evidence for a locally synthesized protein that plays a critical role in synaptic plasticity.

With that, I had discovered data that answered my two previous questions and, most importantly, revealed connections between different steps of the process – from regulating local protein synthesis to the role of locally synthesized proteins in postsynaptic membranes to how synaptic membranes facilitate synaptic plasticity and, finally, the connection to memory. Though the individual cases I examined may not form a single, cohesive pathway, they do collectively provide evidence that all steps of such a pathway exist and are active in neurons. For the purposes of my investigation, this conclusion is sufficient, and gives me hope that there does exist a complete pathway – yet to be observed – which follows a protein all the way from its synthesis in the dendrites to its impact on memory.

Additionally, I came across an article that mentioned "further investigations [which] have identified many other dendritic transcripts, including mRNAs encoding [Glu A1/A2] subunits"(Benson 1997). This sparked my memory, causing me to revisit Henley and Wilkinson's study where AMPARs, though said to be synthesized in the soma, are described as consisting of GluA1/A2 subunits. Considering the difficulty of identifying exactly which proteins are synthesized where in a neuron(Sutton and Schuman 2006), is it out of question that locally synthesized GluA1/GluA2 subunits are used in AMPARs and, if so, AMPARs could be considered locally synthesized to some extent? While an intriguing thought in its own right, this comment also points to some of the most pervasive questions in the research surrounding dendritic protein synthesis and its connection to synaptic plasticity and memory.

As this investigation has shown, one of the most successful approaches in this field of research is to conduct focused case studies, usually on a single protein, mRNA, molecular subunit, etc. As the studies on BNDFs, eCBs, AMPARs and NMDARs demonstrate, tracking the production and function of a specific molecule of interest produces highly detailed explanations for an individual cellular system, the implications of which can then be extrapolated to make broader conclusions. However, one natural limitation of such a narrow approach is that "it is not yet clear what kind of dynamics or rules govern the maintenance, shrinkage, or growth of the synaptic protein interaction matrix"(Sutton and Schuman 2006). As Sutton and Schuman put it, "once we understand whether the molecular memory is due to a few proteins or an emergent property of the system, many of our current isolated observations on individual protein dynamics [and] protein synthesis . . . will appear more unified"(Sutton and Schuman 2006). However, our narrow - though necessary - methods of research seems to provide only a limited understanding of these overarching rules. This is further complicated by the fact that, because the "identification of proteins in dendrites does not indicate their site of synthesis. . . there have been no systemic and unambiguous inquiries to determine the local proteome"(Sutton and Schuman 2006), which refers to all the proteins that are produced locally(Freeman et al. 2017). Thus, if a conclusion hangs on the analysis of a few proteins versus an entire protein system, we have yet to uncover a technique useful in analyzing the latter.

Another gap in knowledge is rooted in the assumption which underlies this entire field of study, which is that the purpose of local protein synthesis is to provide "an elegant mechanism for spatially restricting gene expression within the neuron, such that individual synapses could independently regulate their morphology and efficacy, in a persistent, protein synthesis-dependent manner, in response to specific stimuli"(Martin and Zukin 2006). However, as

demonstrated by eCB, which functions outside of the cell in which it was synthesized, the idea that local synthesis acts solely for the function of each individual dendrite is sometimes disproven. In reality, it is “not known to what extent newly synthesized proteins are shared among neighboring synapses or whether changes in activity at single synaptic sites are capable of regulating local translation in situ”(Sutton and Schuman 2005). Hence, a central question for this field going forward regards the specificity of local protein synthesis in dendrites (Sutton and Schuman 2006). And, if this foundational assumption must be altered or expanded to accommodate the data, what does that mean for this field of research and the conclusions it has drawn thus far?

In short, local protein synthesis in neuronal dendrites proves to be a complex topic, continuously testing the ability of researchers to draw conclusions from the tools available to them at this point in time. However, isolated case studies have revealed the following: local protein synthesis is one of the keys to synaptic plasticity as it allows for specific, efficient response on the part of the dendrites to change the composition of postsynaptic membranes, which is the biological basis for synaptic plasticity and memory. Proteins such BNDF are used by neurons to regulate the localization of molecular machinery responsible for protein synthesis, which both enables and controls local synthesis in the dendrites. These locally synthesized molecules, such as eCB and NMDARs, interact with the cell membrane, each other, and other neighboring cells, hinting at a complex system of localized proteins responsible for responding to environmental stimuli. Ultimately, though, much is not yet understood and many mysteries remain unsolved in this area of research; crucially, it is these knowledge gaps - recognized by researchers - that provide an exciting, uncharted trajectory for further exploration.

References

Benson, D. L. 1997. "Dendritic Compartmentation of NMDA Receptor MRNA in Cultured Hippocampal Neurons." *Neuroreport* 8 (4): 823–28. https://doi.org/10.1097/00001756-199703030-00004.

Cajigas, Iván J., Georgi Tushev, Tristan J. Will, Susanne tom Dieck, Nicole Fuerst, and Erin M. Schuman. "The Local Transcriptome in the Synaptic Neuropil Revealed by Deep Sequencing and High-Resolution Imaging." *Neuron* 74, no. 3 (May 10, 2012): 453–66. https://doi.org/10.1016/j.neuron.2012.02.036.

Duarte, Carlos B. "BDNF-Induced Local Protein Synthesis and Synaptic Plasticity," January 27, 2021. https://core.ac.uk/reader/19630132?utm_source=linkout.

Fonkeu, Yombe, Nataliya Kraynyukova, Anne-Sophie Hafner, Lisa Kochen, Fabio Sartori, Erin M. Schuman, and Tatjana Tchumatchenko. "How MRNA Localization and Protein Synthesis Sites Influence Dendritic Protein Distribution and Dynamics." *Neuron* 103, no. 6 (September 2019): 1109-1122.e7. https://doi.org/10.1016/j.neuron.2019.06.022.

Freeman, Scott, Kim Quillin, Lizabeth A. Allison, Michael Black, Greg Podgorski, Emily Taylor, and Jeff Carmichael. *Biological Science*. Sixth edition. Boston: Pearson, 2017.

Hegde, Ashok N., and Spencer G. Smith. "Recent Developments in Transcriptional and Translational Regulation Underlying Long-Term Synaptic Plasticity and Memory." *Learning & Memory* 26, no. 9 (September 2019): 307–17. https://doi.org/10.1101/lm.048769.118.

Henley, Jeremy M., and Kevin A. Wilkinson. "AMPA Receptor Trafficking and the Mechanisms Underlying Synaptic Plasticity and Cognitive Aging." *Dialogues in Clinical Neuroscience* 15, no. 1 (March 2013): 11–27.

Jewett, Benjamin E., and Sandeep Sharma. "Physiology, GABA." In *StatPearls*. Treasure Island (FL): StatPearls Publishing, 2020. http://www.ncbi.nlm.nih.gov/books/NBK513311/.

Jiang, Changan, and Erin M Schuman. "Regulation and Function of Local Protein Synthesis in Neuronal Dendrites." *Trends in Biochemical Sciences* 27, no. 10 (October 1, 2002): 506–13. https://doi.org/10.1016/S0968-0004(02)02190-4.

Klann, Eric, and Thomas E. Dever. "Biochemical Mechanisms for Translational Regulation in Synaptic Plasticity." *Nature Reviews Neuroscience* 5, no. 12 (December 2004): 931–42. https://doi.org/10.1038/nrn1557.

Martin, Kelsey C., and R. Suzanne Zukin. “RNA Trafficking and Local Protein Synthesis in Dendrites: An Overview.” *Journal of Neuroscience* 26, no. 27 (July 5, 2006): 7131–34. https://doi.org/10.1523/JNEUROSCI.1801-06.2006.

Khan Academy. “Overview of Neuron Structure and Function (Article).” Accessed February 14, 2021. https://www.khanacademy.org/science/biology/human-biology/neuron-nervous-system/a/overview-of-neuron-structure-and-function.

Khan Academy. “Overview of Translation (Article).” Accessed February 14, 2021. https://www.khanacademy.org/science/ap-biology/gene-expression-and-regulation/translation/a/translation-overview.

Sutton, Michael A., and Erin M. Schuman. “Dendritic Protein Synthesis, Synaptic Plasticity, and Memory.” *Cell* 127, no. 1 (October 2006): 49–58. https://doi.org/10.1016/j.cell.2006.09.014.

Sutton, Michael A., and Erin M. Schuman. 2005. “Local Translational Control in Dendrites and Its Role in Long-Term Synaptic Plasticity.” *Journal of Neurobiology* 64 (1): 116–31. https://doi.org/10.1002/neu.20152.

Swanger, Sharon A., and Gary J. Bassell. “Dendritic Protein Synthesis in the Normal and Diseased Brain.” *Neuroscience* 232 (March 1, 2013): 106–27. https://doi.org/10.1016/j.neuroscience.2012.12.003.

Tang, Shao Jun, and Erin M Schuman. “Protein Synthesis in the Dendrite.” *Philosophical Transactions of the Royal Society B: Biological Sciences* 357, no. 1420 (April 29, 2002): 521–29. https://doi.org/10.1098/rstb.2001.0887.

Younts, Thomas J., Hannah R. Monday, Barna Dudok, Mathew E. Klein, Bryen A. Jordan, István Katona, and Pablo E. Castillo. “Presynaptic Protein Synthesis Is Required for Long-Term Plasticity of GABA Release.” *Neuron* 92, no. 2 (October 19, 2016): 479–92. https://doi.org/10.1016/j.neuron.2016.09.040.