# Nascent Proteins in Activity-Induced Cortical Glutamatergic Neurons

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**Received**: 4-Oct-2022, Manuscript No. NCOA-22-77895; **Editor assigned:** 6-Oct-2022 PreQC No. NCOA-22-77895 (PQ); **Reviewed**: 15-Oct-2022, QC No. NCOA-22-77895 (Q); **Revised**: 18-Oct-2022, Manuscript No. NCOA-22-77895 (R); **Published**: 25-Oct-2022, doi. 10.4172/2469-9780.2022.8.5.186

## Abstract

Neuronal activity triggers communication cascades that lead to a variety of effects, such as metabolic changes and structural and functional plasticity in neurons. Despite studies showing activity-dependent, cell type-specific transcriptional changes in neuronal cells, cellular heterogeneity and a relatively low abundance of NSPs in the brain's proteome have made it difficult to conduct unbiased quantitative analyses of cell-specific activity-induced dynamics in Newly Synthesized Proteins (NSPs) synthesis in vivo. Here, we paired tightly timed treatment with the no canonical amino acid azidonorleucine to biotinylated NSP with targeted production of mutant MetRS (methionine tRNA synthetase) in genetically specified cortical glutamatergic neurons.

## Introduction

Activity induces the production of proteins that are thought to have homeostatic functions in response to activity, including proteins that regulate proteostasis, control intracellular ions, and remodel the cytoskeleton. On the other hand, seizure reduced freshly synthesized NCAM and possibly other molecules, indicating that seizure triggered degradation. In order to identify downstream mediators of neuronal plasticity and develop ideas about their role, we determined quantitative alterations in the activity-induced nascent proteome from genetically specified cortical glutamatergic neurons.

However, it has proven technically difficult to identify neuronal cell type-specific nascent proteome dynamics in the intact brain. Activity-induced newly synthesized proteins are one type of protein that changes in the protein landscape that enable activity-induced neuronal and synaptic plasticity. Within 20 hours of a pharmacologically produced seizure, we performed an unbiased proteomic screen and found significant activity-related alterations in newly generated proteins in genetically defined cortical glutamatergic neurons. Our understanding of the variety of plasticity mechanisms is expanded by bioinformatics analysis of the dynamic nascent proteome, which shows that the newly synthesized proteins play a variety of roles in excitatory and inhibitory synaptic plasticity, chromatin remodeling, homeostatic mechanisms, and proteasome and metabolic functions.

Brain function depends on activity-driven plasticity in cortical neurons. Our limited understanding of brain disorders exposes fundamental knowledge gaps on the alterations to the brain caused by activity. Following seizures, traditional research identified activity-induced plasticity genes, but more recent analysis of activity-regulated transcriptional programmers unique to different cell types revealed the range of activity-dependent processes.

Studies have also shown that increasing brain activity causes proteome alterations, although it is still unclear how activity-regulated newly synthesized proteins (NSPs) from specified neural cell types are identified and characterized. Following a pentylenetetrazol (PTZ)-induced seizure, we investigated this issue by performing an impartial screen of differentially expressed NSPs in cortical glutamatergic neurons.

Proteomic approaches can reveal additional activity-responsive proteins and signaling pathways, commensurate with the complexity of the proteomic landscape, according to recent mass spectrometry-based proteomic studies that identified activity-induced NSPs, including novel candidate plasticity proteins induced in response to visual experience. Bio-orthogonal ncAA tagging, also known as labelling NSPs with azide-containing No Canonical Amino Acids (ncAA) methionine analogues paired with click chemistry conjugation to alkynes, has been a significant method for studying protein dynamics.

The development of efficient recovery techniques and direct detection of biotin-modified peptides, known as DiDBiT, improved BONCAT for the identification and measurement of developing proteomes in vivo. In these findings, the ncAA Azidohomoalanine (AHA) was incorporated into NSPs in all cell types via natural translational machinery. For example, production of a Mutant Methionine Trna Synthetase (mMetRS) that only charges the methionine analogue Azidonorleucine (ANL), which cannot be integrated into proteins by endogenous translational machinery, restricted the integration of ncAA into genetically targeted cells. These methods allow the analysis of cell type-specific NSPs in intact animals, but because the labelling procedures used in these studies took several weeks, it's possible that they cumulatively labelled "baseline" NSPs before they were exposed to stimuli that caused plasticity, making it difficult to detect these NSPs.

In activity-induced NSPs, we aimed to measure alterations in cell type. Within 20 hours of a PTZ-induced seizure in adult mice, we produced mMetRS in cortical glutamatergic neurons using EMX-cre, and we discovered ANL-labeled NSPs. First, we showed that mMetRS expression has no negative effects on animal behaviour or development. Then, we made a number of adjustments to the techniques for NSP labelling and analysis. Our experimental design for PTZ and ANL treatments, as well as tissue collection, was influenced by our optimization of the temporal regulation of ANL administration in the brain. To improve NSP identification and quantitation, we combined DiDBiT with tagging PTZ and control samples with heavy and light biotin-alkynes. We contrasted ANL-labeled NSPs from mMetRS-expressing mice with baseline AHA-labeled NSPs. Proteins from many types of brain cells were included in AHA-labeled NSPs.

About 300 NSPs associated to synaptogenesis, cytoskeletal dynamics, GTPases, and G-protein-coupled receptors were dramatically impacted by PTZ. Additionally, PTZ enhanced nuclear NSPs that control chromatin remodeling, such as Rad21 and SMARCA2. As a result of the richness of our collection, it was possible to use ChipSeq databases to find upstream NSP regulators, providing both retrospective and prospective data on activity-dependent proteomic alterations in cortical excitatory neurons in intact animals. We were able to improve the spatial and temporal labelling of activity-induced NSPs with cell-type resolution, enrichment, and direct tandem Mass Spectrometry (MS/MS) detection of biotin-tagged peptides, as well as a better quantitative approach using heavy and light tags, all as a result of our original experiment. We improved the signal-to-noise in our proteome datasets by combining the DiDBiT workflow, sample multiplexing, and heavy and light isotopic tags.

It is well known that activity-induced protein synthesis acts as a mechanism to enhance synaptic and cellular plasticity in neurons. Therefore, changes in NSPs involved in synaptic plasticity, as well as the maintenance and stabilization of plastic synapses, are anticipated to be revealed by activity-dependent proteome dynamics.

Although it is well recognized that increasing neuronal activity results in significant alterations in protein synthesis, little is understood regarding the regulation, extent, and function of activity-induced NSP dynamics. A useful method to explore the signaling and regulatory cascades that are started and reinforced by these increases and decreases in new protein synthesis that are brought on by elevated neuronal activity is bioinformatics pathway analysis.

In order to find NSPs generated in vivo in mouse cortical glutamatergic neurons within 20 h after a PTZ-induced seizure, we employed an improved workflow for unbiased proteomic detection of NSPs in genetically specified neurons. We demonstrated that labelling NSPs with AHA or ANL is equivalent using HEK cells. We demonstrated that EMXcre-driven mMetRS expression has no significant impact on either brain structure or behavioral plasticity. Based on the pharmacokinetic profile of intraperitoneally administered ANL in the brain, we optimized the temporal resolution of labelling activity-induced NSPs in vivo. We used heavy or light biotin-alkyne to tag ANL-labeled NSPs and tandem mass spectrometry to treat samples using DiDBiT.

**Cite this article:** Hollis, M. Nascent Proteins in Activity-Induced Cortical Glutamatergic Neurons. J Neurosci Neuropharmacol. 2022, 8.5, 001-002.