# RPL7 silencing affects neurite development

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**Abstract**

Preliminary research done in the Rasin Lab has indicated that RPL7 plays a role in the early stages of neocortical development, most notably in determining neuronal morphology. Here, our objective was to characterize the role that RPL7 plays in the development of neurons, specifically examining DIV4 and DIV8 neurons in control and RPL7 knockdown. Researchers performing this experiment were blinded, so groups are referred to as GFP and RFP. While the current sample size is inadequate in determining a statistically significant result, either for or against the hypothesis, early data suggest differences between the two groups of neurons. As it stands, more data will need to be collected, in a more efficient and effective manner, as well as in a way that ensures enough data is being collected for all groups.

**Introduction**

The mammalian neocortex is the primary system responsible for advanced cognitive functions, such as sensation, voluntary motor function, and spatial reasoning (Rakic, 2009). These functions are able to be fulfilled due to the structure of the functional neocortex. The architecture of the neocortex, as well as that of the neurons that comprise it, is in some way tied to the cognitive abilities that it grants, and as a result, its development is similarly important to overall cognition. Normal neocortical development is characterized by spatiotemporal birth, migration, and differentiation (Popovitchenko and Rasin, 2017). Defects in neocortical development has been tied to

several neurological dysfunctions, notably autism spectrum disorders (Chomiak and Hu, 2013).

Neocortical neurons are organized in six layers that develop from the innermost layer to the outermost layer in a characteristic lamination that is critical in ensuring proper neurological function (Isaac and Feldmeyer, 2009; Popovitchenko and Rasin, 2017). Lamination is achieved by differentiation of neurons from progenitors in the ventricular zone (Bystron, Blakemore, and Rakic, 2008), which subsequently migrate radially to form the outermost layer. These descendants undergo further maturation and project themselves, forming circuits. The six layers of the neocortex are distinct from each other, and can be distinguished by their morphology, gene expression, and projection, so much so that they can be classified as “lower layer” (LL, V-VI) or “lower layer” (UL, II-IV).

These two categories of layers possess neurons that project themselves in clearly different ways. LL neurons project below the cortex, connecting to central structures including the brainstem and spinal cord while UL neurons project within the neocortex forming local circuits instead (Belgard et al., 2011). The morphology of the neurons themselves also vary by layer, including clear differences in dendritic arborization between neurons in the UL and LL (Petreanu et al., 2009), which has been associated with differences in gene expression between the two types of layers. Previous studies have indicated sets of transcription factors that are expressed exclusively in either UL or LL neurons (Belgard et al., 2011).

The expression of these specific genes, and just as importantly translation of the resultant proteins, are critical in morphological development. The ribosome, made up of

ribosomal proteins (RPs), is the structure responsible for translation. Recent studies have demonstrated that the ribosome plays a more dynamic role in regulation of transcript expression, as opposed to its historical static mechanism (Xue and Barna, 2012). The dynamic nature of this regulation could likely be due to the apparent "preference" that different RPs have for certain populations of mRNAs (Shi et al., 2017). The role of RPs has also been tied to tissue specification (Guimaraes and Zavolan, 2016), suggesting that RPs may be a major factor in neocortical development.

Preliminary data suggests that one particular ribosomal protein, RPL7, shows a preference for associating polysomes found in the lower layers, indicating increased translation in the basal layers of the neocortex as compared to UL neurons. Polysomes are sites of active translation, and RPL7's heightened association with them in the lower layers compared to the upper layers suggests that RPL7 plays some context-specific role in the lower layers. Prior experiments have shown that overexpression of RPL7 results in increase of dendritic length as well as abnormal projection of axons into regions of the subcortex. However, the exact mechanisms of RPL7 in regulating expression dynamically, as well as the effects of RPL7 knockdown are still unclear. Therefore, we hypothesize that RPL7 promotes the growth of neurites in the lower layers within the developing neocortex.

## Materials and Methods

**Prior procedures and prepared materials.** Primary cell cultures were prepared by dissecting brain regions from embryonic mice, mincing them, and centrifuging. Then cells

were plated onto coverslips that had been previously incubated. Immunocytochemistry was done at 4 and 8 days *in vitro* to bind primary and secondary antibodies for imaging. DAPI was used to stain cell nuclei, while RFP, and GFP were utilized as coinjection markers. Western blotting was performed to confirm RPL7 knockdown in experimental group cells.

**Imaging and reconstruction**. Confocal microscopy was used to image neuronal systems. Z-stack images were taken of candidate neurons. Channel signal strengths were calibrated and standardized to best fit all culture slides. Images were sent to Neurolucida for reconstruction. The axon was defined as the longest projection outwards from a cell body. Neurolucida Explorer was then used to generate neuronal data as well as Sholl analysis reports.

**Data analysis**. While n values were too small to generate any meaningful t-test results for axonal length and dendritic complexity from the Sholl analysis. Due to the time constraints and the time required to image each individual neuron, no characteristics were examined for significance, although data will continue to be gathered. It is important to note that the researchers in this particular stage of the experiment were blinded to the specific groups that constitute the cell cultures, so they will be reported as “GFP” and “RFP.”

## Results

### Early data suggest differences in dendritic complexity.

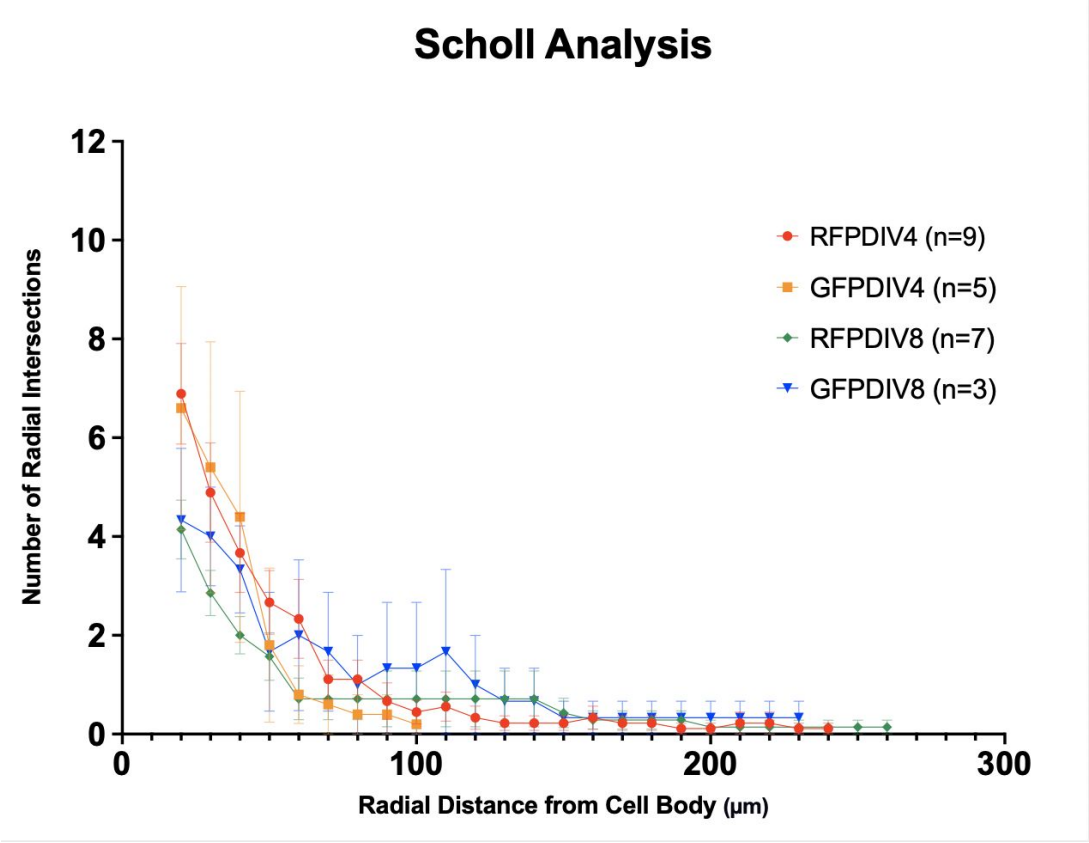


***Figure 1.*** *Sholl analyses of DIV4 and DIV8 neurons for both RFP and GFP. Researchers were blinded to the groups associated with RFP and GFP. Statistical analysis was not done for this data.*

Preliminary data seems to suggest some differences in the overall complexity of developing neurons. RFP in DIV4 seems to show increased numbers of radial intersections at around 75 µm from the cell body, but in DIV8, this trend seems to reverse towards GFP, which shows a heightened radial distance around 100 µm. However, statistical analysis was conducted, so more data must be collected to draw a statistical conclusion.

**Early data suggest differences in axon length.**

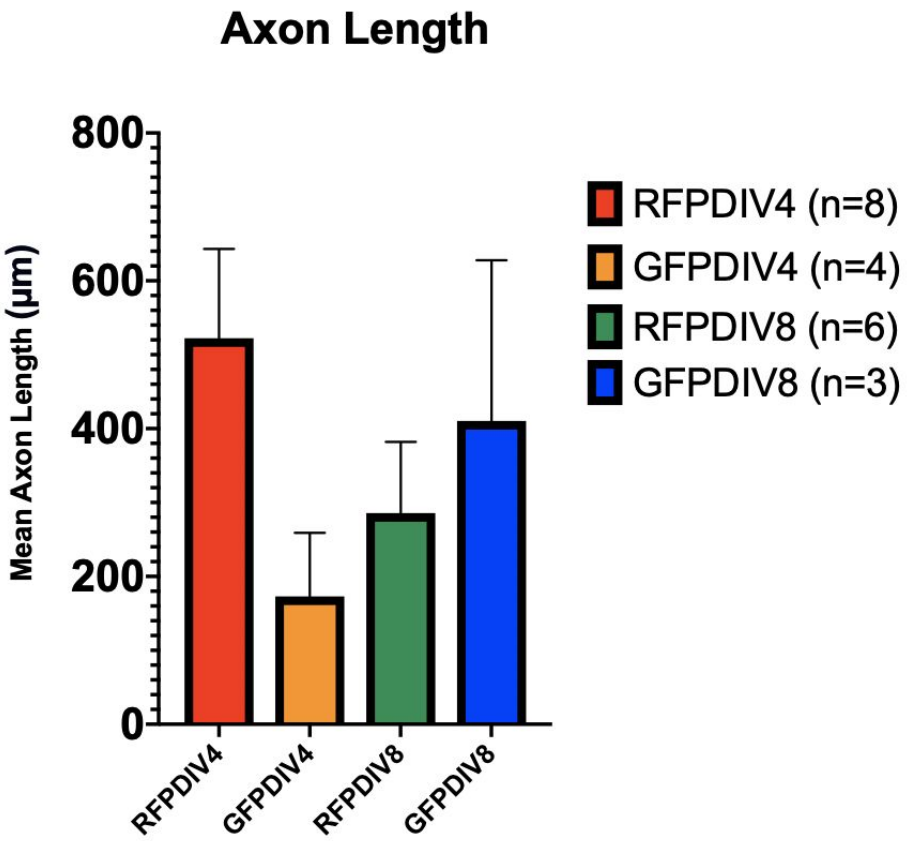

***Figure 2.*** *Mean axon length for GFP and RFP neurons for both DIV4 and DIV8. Researchers were blinded to GFP and RFP associated groups. No statistical tests were run on this data.*

Early data seems to indicate a difference in the mean axon length of developing neurons, but between DIV4 and DIV8, this difference seems to reverse. Although the exact significance of these differences remains to be calculated, more data will be collected and statistical conclusions will be drawn later.

**Discussions**

This study is intended to continue ongoing experiments concerning RPL7 and its role in neocortical development in the embryo. Specifically, this experiment examines neuron morphology between control groups and RPL7 knockdown groups at DIV4 and DIV8, notably because DIV4 represents early stage neuronal development, while DIV8 represents late neuronal development. This will allow us to further characterize RPL7's role as a temporally dynamic regulator.

Although more data will have to be collected in order to make a draw a truly significant conclusion, the current trend of data seems to point towards early signs that there is indeed some difference between GFP and RFP neurons. Which group refers to the control and which group refers to the RPL7 knockdown is unknown to the researchers to maintain blindness, but there certainly seems to be some differences in axon length and dendritic complexity between GFP and RFP, although the differences reverse towards the

other group when comparing between DIV4 and DIV8. This suggests an additional temporal role that RPL7 might play in development, although this will have to be examined more closely by taking additional time points within the experiment.

These results seem to align with prior literature and previous research done. Prior literature has suggested the role of RPs in differentiation of cells (Guimaraes and Zavolan, 2016), and prior tests in this experiment has shown RPL7 overexpression to have increased dendritic length. Currently, data suggest that RPL7 has some direct effect on neuron morphology, although the actual mechanism for this still remains unclear.

Although the current pace of data collection is acceptable, presently, there are minor issues involved in the imaging portion of data collection. These issues stem mostly from technical difficulties and sheer pace of imaging concerned with the confocal microscope used to capture the neuronal systems in the cell cultures being analyzed. The major issue concerning technical errors is concerned with certain channels not being present when attempting to image. In order to counteract this, a “calibration” system was noted in a geographically distinct location on the slide that had systems in both GFP and RFP channels. One slice in the z-stack was taken for this system to ensure that the software would capture all channels necessary for imaging.

It is clear that improvements in this experiment will be concerned with gathering data in a more efficient manner, as well as improving on the methods being used now. One improvement involves imaging an equal amount of both DIV8 and DIV4 neurons, to ensure relative balance of data collected. Another improvement involves improving imaging times by reducing the number of z-slices at the beginning and end of images. In

order to maintain good quality of images, these slices should not include the neuron itself, but rather reduce the "blank space" at the start and end of images, thereby reducing the total time required to image one neuron.

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