Proceedings of the 3rd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/2025.24218

The Role and Relationship of S1P and Reelin in the Horizontal-to-Radial Transitioning of Newly Generated DGCs in the Hippocampus

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Abstract: This work studies the functions of sphingosine-1-phosphate (S1P) and Reelin in the horizontal-to-radial transitioning of freshly formed dentate granule cells (DGCs) in the hippocampus. We determined the location of S1P synthesis in the dentate gyrus, investigated the cooperative character of S1P and Reelin signaling, and looked at the regulatory link between the two. According to our research, the polymorphic layer or granule cell of the dentate gyrus is where S1P is formed. Moreover, we show that the horizontal-to-radial transitioning of DGCs is facilitated by the joint contribution of S1P and Reelin signaling. Crucially, our findings imply that Reelin participates in this developmental process upstream of S1P. These discoveries deepen our knowledge of hippocampus neurogenesis and could have consequences for neurological conditions linked to abnormal.

Keywords: Neurogenesis, Hippocampus, Dentate Gyrus

1. Introduction

Reelin is an extracellular matrix protein. The pathway of reelin has an immense influence on the maturation as well as integration of early postnatal and adult-generated DGCs. The pathway of reelin in the mature hippocampal area controls the migration and dendritic development of the neurons of the hippocampus. Overexpression of Reelin accelerates dendritic maturation, while disruption of the pathway of Reelin results in abnormal synaptic circuit formation and abnormalities in the growth and orientation of the dendrites.

Up to now, no investigations have been able to prove that S1P and reelin signals perform the same functions in the migration of newly generated DGC. However, since they are involved in the regulation of the movement of cells, the two can influence this process through similar mechanisms.

S1P controls the realignment of newly formed DGCs by regulating cell migration. More recently, studies have found that dysregulated expression of reelin itself contributes to the pathogenesis of diseases such as AD and epilepsy. Reelin readjusts the new DGC through its receptor and intracellular cascade activation.

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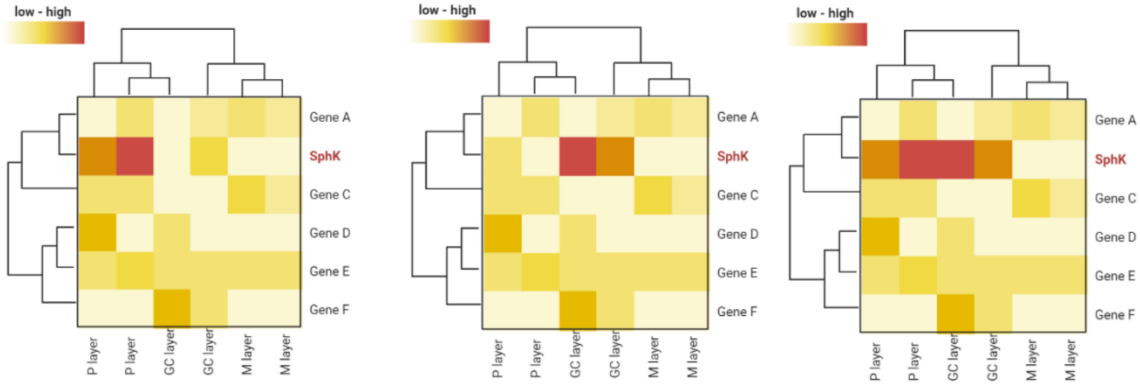
2. Results

2.1. Sphingosine-1-phosphate is produced in the granule or polymorphic layer of the dentate gyrus

The horizontal to radial repositioning of newly produced dentate granule cells relies on sphingosine-1-phosphate. As previous studies indicate, S1P is produced by resident cells of the dentate gyrus and secreted extracellularly to aid the new cells in settling in place. Although an important function of the dentate gyrus has been established, the specific cell type or layer that produces S1P in the dentate gyrus remains unknown. Before answering this question, we need to understand the synthesis pathway of S1P. The starting molecule for S1P synthesis is sphingosine. Sphingosine kinase catalyzes the phosphorylation of sphingosine to yield the S1P product [4]. The expression level of the sphingosine kinase gene will then be assessed to pinpoint the precise layer of the dentate gyrus or the particular cell type producing S1P. Those cells expressing high levels of sphingosine kinase will help in the precise location. S1P Synthesis.



(a) (b) (c)

Figure 1: (a) Polymorphic layer; (b) granule cell layer; (c) polymorphic layer, granule cell layer

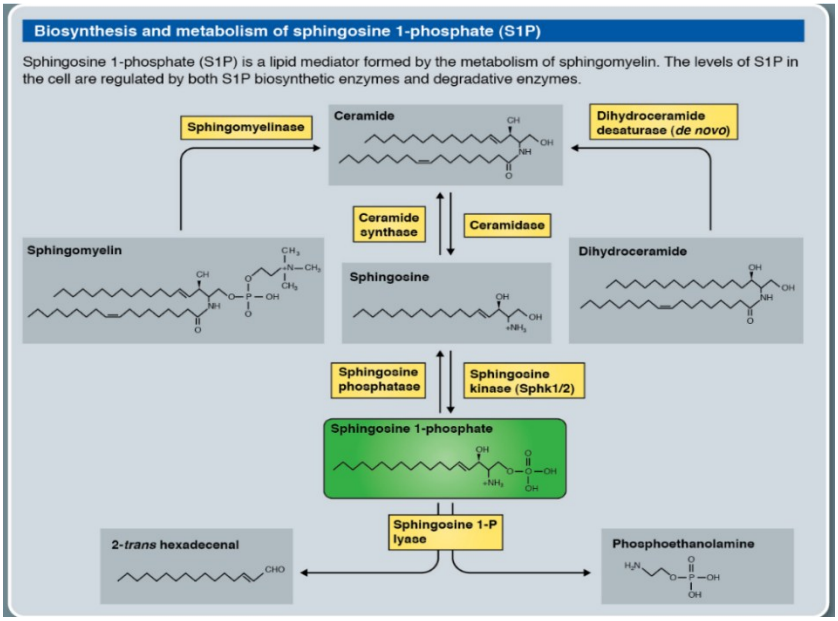


Figure 2: Biosynthesis and metabolism of S1P

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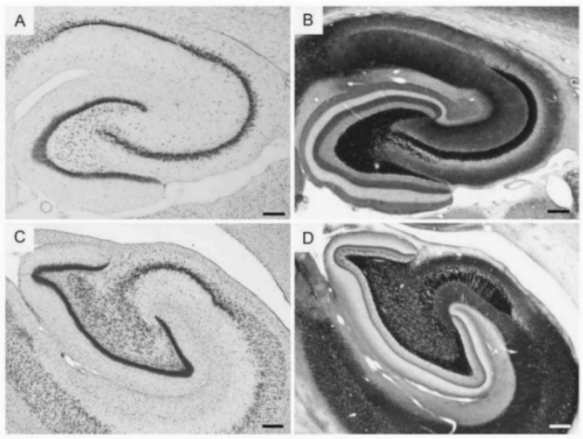

Figure 3: Dentate gyrus organization

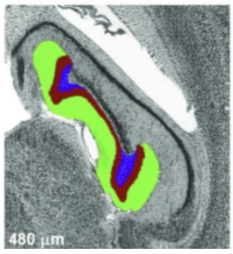

Figure 4: The subgranular zone

The dentate gyrus is made up of three layers [3]. These are the inner polymorphic layer, the middle granule cell layer, and the outside molecular layer. Neurogenesis occurs within the subgranular zone between the granule cell layer and the polymorphic layer [1]. S1P could be produced in the granule cell layer, the polymorphic layer, or both. From our data, we will be able to see that the dentate gyrus areas that are making S1P will have increased levels of sphingosine kinase. By looking at this area, we can also ask whether reelin and S1P signaling play a cooperative or independent role in the horizontal-to-radial transition of the DGCs in this region.

2.2. Reelin and S1P signaling contribute to the horizontal-to-radial transitioning of newly generated DGCs cooperatively

We formulate the question: Do Reelin and S1P signaling contribute cooperatively or independently to the horizontal-to-radial transitioning of freshly created DGCs? This is based on experiment one, where we established that s1p is produced in the subgranular zone of the dentate gyrus. Do they share a pathway with each other? We established three experimental groups in order to address this. Reelin, S1PR1, and Reelin and S1PR1 together were all taken out by us. The DGC phenotypes for each group were then assessed. Reelin and S1P may be involved in the same pathway if the phenotypes were comparable. We first used inducible shRNA vectors encoding the knockdown sequences for Reelin, S1PR1, and both Reelin and S1PR1 to infect our mice for our flow scheme. In order to induce shRNA expression, we added doxycycline to the drinking water three days after the infection. The phenotypes at 5, 7, and 21 days after infection were then examined. We used immunostaining to evaluate the knockdown efficiency.

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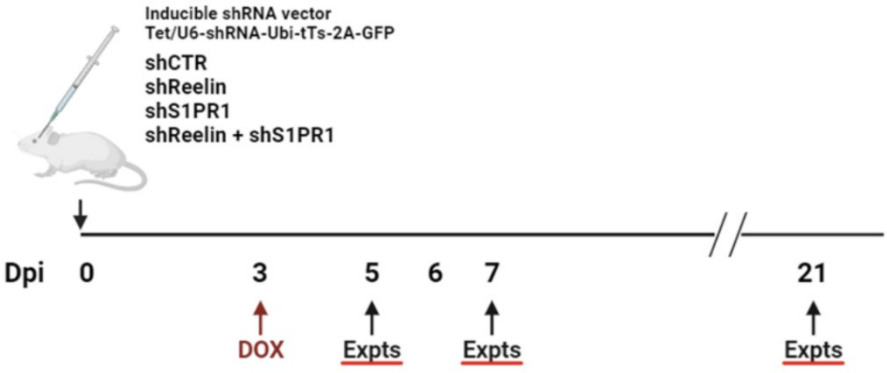

Figure 5: Flow scheme of mice experiment

The immunostaining data for S1PR1 and Reelin are displayed in Figure A. Reelin and S1PR1 expression is reduced in the bottom two rows, while the top two rows use luciferase to represent the control group. The Reelin expression (20–30%) has significantly decreased, as shown by Graphs B and C. At 4 and 7 days after infection, we looked at the morphological maturation of DGCs under four different circumstances. With dendritic growth, cells in the control group shifted from a horizontal to a radial orientation [4,6]. The other three groups, however, barely changed at all. Seven days after infection, we saw a normal decline in the number of horizontal cells in the control group, as shown in Graph E. This suggests a change from horizontal to radial. In addition, we counted the number of neurites and discovered that control DGCs had greater neurite development than the knockdown groups. In order to evaluate synaptic transmission, we measured excitatory postsynaptic currents 21 days after infection using electrophysiological recordings, as shown in Graph F. Considering their increased development, the control DGCs displayed a greater synaptic integration frequency. We can see that the knockdown groups have comparable phenotypes from Figures D, E, and F. All of them are horizontal cells with limited neurite expansion. Given that Reelin and S1P signaling exhibit comparable symptoms upon knockdown, these similarities imply that they participate in the same pathway.

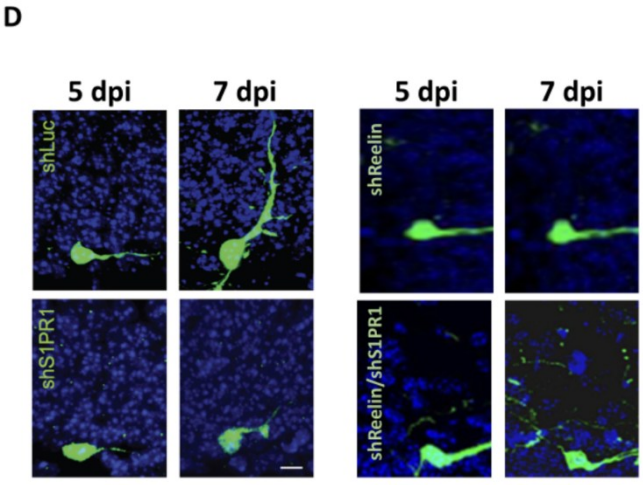

Figure 6: Angle distribution of shS1PR1+ DGCs

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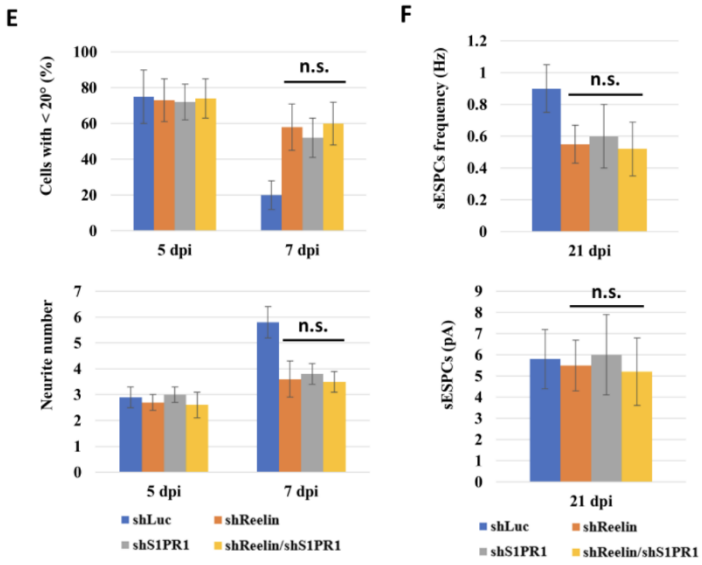

Figure 7: DGC expression of vectors

2.3. Reelin regulates upstream of S1P during the horizontal-to-radial transitioning of newly generated DGCs in the hippocampus

Our third research question is formulated based on our first two experiments: Does Reelin influence S1P signaling during the hippocampus's freshly formed DGCs' horizontal-to-radial transition, and if so, does Reelin function ahead of S1P throughout this developmental process? To examine the role of Reelin in regulating S1P signaling during the horizontal-to-radial transition of newly born dentate granule cells (DGCs) in the hippocampus, we took a retroviral knockdown approach. Short hairpin RNA (shRNA) that binds to Reelin mRNA was introduced into hippocampal cells via a modified retrovirus to effectively inhibit Reelin protein production. Following Reelin knockdown, we measured the levels of S1P for the effect elicited on S1P signaling. Quantitative assay techniques were employed to quantify S1P levels in hippocampus tissues [5]. We checked for the behavior of newly generated DGCs, seeing if they would shift from horizontal to radial orientation following Reelin knockdown. To understand the movements and morphological changes of DGCs, real-time imaging and tracking of cells were required. Through monitoring changes in gene expression and cellular activity, we performed analyses to understand the downstream effects of Reelin knockdown. Regarding this, the mRNA expression levels of genes associated with S1P signaling and associated pathways were analyzed. RNA sequencing was performed to study transcriptome effects in Reelin knockdown hippocampal cells. RNA-seq allowed us to quantify the amount of different mRNA molecules, thus providing information on changes in gene expression mediated by Reelin knockdown. We performed reciprocal experiments to confirm the upstream-downstream relationship between Reelin and S1P. We not only knocked down Reelin and saw how it affected S1P signaling, but we also changed S1P levels and looked at how it affected Reelin signaling pathways. This two-way strategy contributed to the validation of the Reelin-S1P regulatory hierarchy. Under our hypothesis, the knockdown of Reelin would lead to reduced S1P signaling and failure of the DGCs to transition from a horizontal to radial alignment. Together, these results indicate that Reelin acts in this developmental process prior to S1P and that Reelin signaling is required for the proper regulation of S1P.

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3. Discussion

Higher expression levels of sphingosine kinase in the granule cell layer and the polymorphic layer of the dentate gyrus indicated by our initial experiment that S1P is generated in these regions. These layers are located in the subgranular zone where neurogenesis takes place, which is compatible with S1P's function in promoting the placement of new cells. Determining the precise locations of S1P production sites provides a basis for comprehending the dynamics of signaling molecules that are involved in DGC formation.

Our knockdown experiments provide strong evidence that Reelin signaling and S1P cooperate to drive the horizontal-to-radial transition of newly born DGCs. Reelin, S1PR1, and double Reelin/S1PR1 knockdown groups performed similarly, suggesting that these signaling pathways likely form part of the same developmental cascade. This finding is important because it shows how many different signaling molecules interact in complex ways to regulate the development of neurons.

Our third experiment showed that Reelin is responsible for the horizontal-to-radial switching of DGCs upstream of S1P. This link is further supported by the reduced S1P signaling along with the defective transition following Reelin knockdown. This observation shows some of the detailed mechanisms for DGC generation and emphasizes the critical role that Reelin assumes in the neurogenesis program within the hippocampus.

Such knowledge on S1P-Reelin signaling in the development of the DGCs would go a long way in providing meaningful insights into several neurological disorders. Previous studies have identified that the differential expression of reelin is associated with a wide range of diseases that include, but are not limited to, Alzheimer's disease and epilepsy. Our findings point to a suspicion that one of the reasons for such disorders may be due to an abnormal neuronal migration and integration caused by alterations of this signaling cascade.

Although our study provides some useful information, it also bears its limitations. For instance, knockdown approaches hardly capture the complexity of how genes function in vivo. Such studies would require more specific genetic approaches, including the use of the recent CRISPR-Cas9 gene editing tool, for a complete explanation of the functions of S1P and Reelin.

Understanding the molecular mechanism underlying DGC development will be enhanced when research has focused on its downstream targets of the S1P-Reelin pathway. Potential treatments which act by inhibiting this pathway would give promise to neurological diseases linked to dysfunctional hippocampal neurogenesis.

4. Conclusion

Herein, this study underlines the critical roles of S1P and Reelin during the transitioning of hippocampal DGCs from horizontal to radial. Our findings confirm that the dentate gyrus produces S1P and that S1P and Reelin signaling pathways work together in a cooperative manner to facilitate this developmental transition. More importantly, we demonstrate that Reelin, in an upstream manner, defines a regulatory hierarchy critical for proper DGC development of S1P. The provided findings expand our current understanding of hippocampus neurogenesis and further raise the possibility of neurological conditions in relationship to aberrant DGC formation. Future study focusing on the downstream effects that have been caused by S1P and Reelin signaling should be the prime focus, given that this will lead to more focused approaches of treatment against diseases like epilepsy and Alzheimer's.

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