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Supplemental Information

**Circuit Integration Initiation
of New Hippocampal Neurons
in the Adult Brain**

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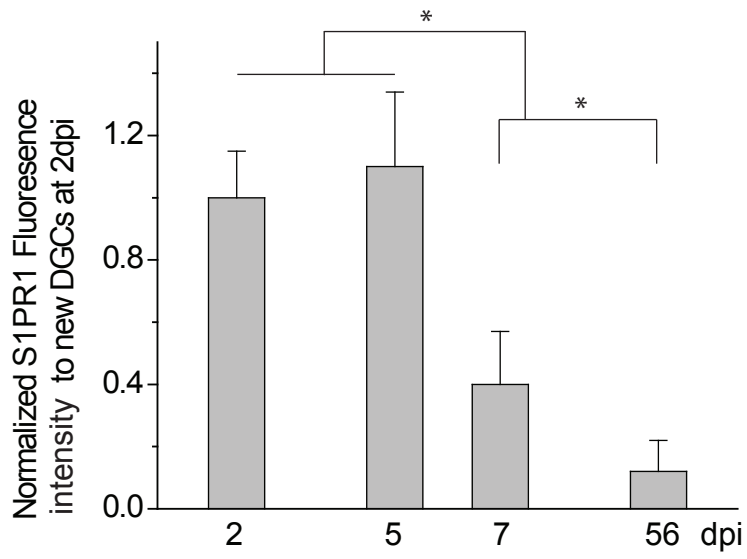


Figure S1. S1PR1 expression profile along DGC development (related to Figure 2). Normalized fluorescence intensity of S1PR1 signal at various time points. One-way ANOVA followed by post-hoc LSD tests, * $P < 0.05$. (n = 30 cells from 3-6 mice each condition)

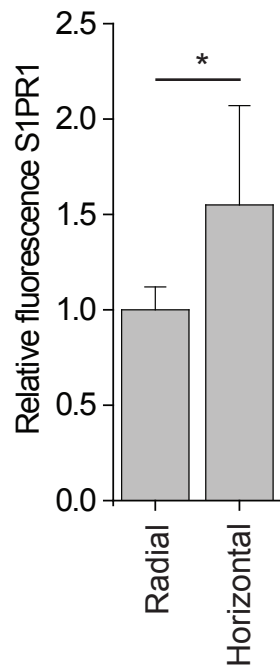


Figure S2. Radial new DGCs express lower levels of S1PR1 compared to horizontal ones at 7 dpi (related to Figure 2).

Normalized fluorescence intensity of S1PR1 signal comparing horizontal-to-radial GFP-labeled new DGCs at 7dpi. Unpaired t-test, *P < 0.05. (n = 18 cells from 3 mice).

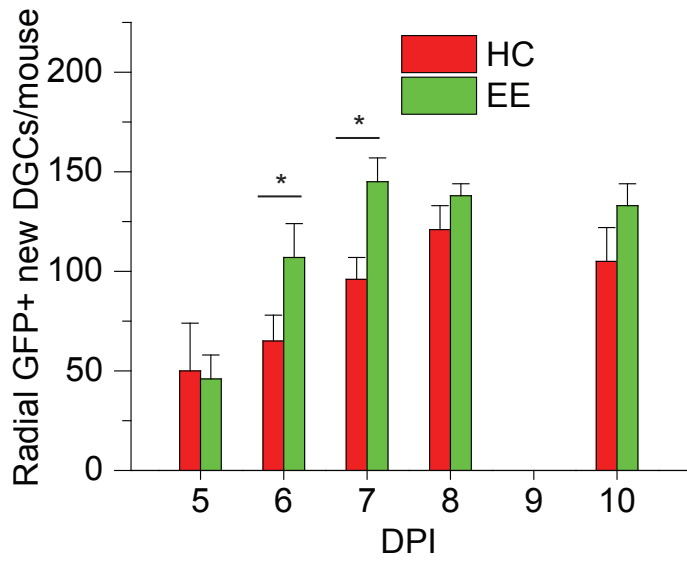


Figure S3. Number of radially positioned new DGCs (related to Figure 4).

The numbers of radially positioned GFP+ DGCs at 5, 7, 8, 10 dpi were counted from cohorts of 5–9 mice at each stage under HC and EE conditions. *P < 0.05 (one-way ANOVA). (n = 40-50 cells from 3-4 mice).

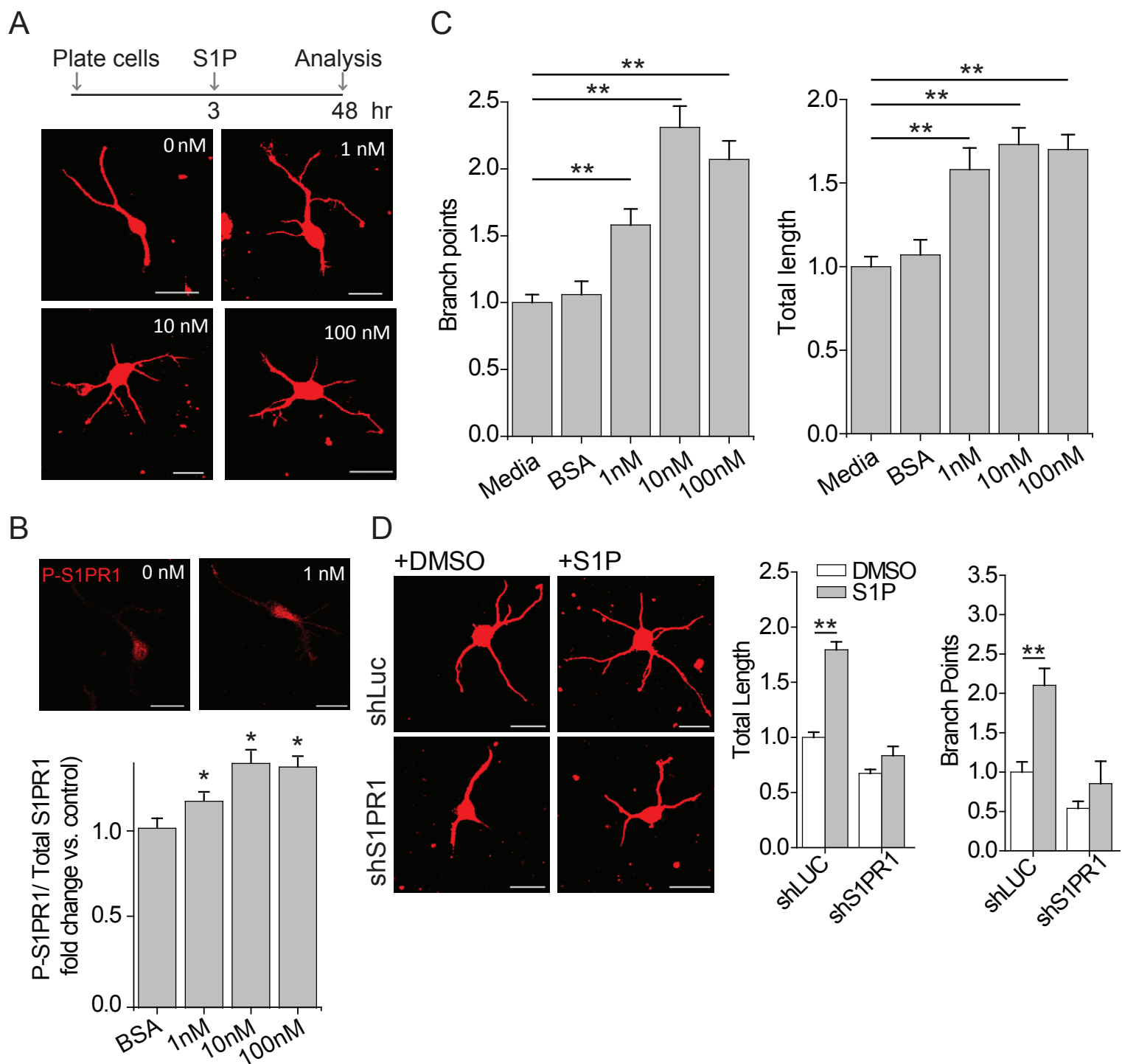


Figure S4. S1P increases dendritic outgrowth of cultured DGCs (related to Figure 4).

A) (Top) Experimental procedure of plating E18 hippocampal cells, adding S1P (or medium or BSA only as controls), and fixing cells at 48 h in vitro. (Bottom) Representative images of cultured cells from the various conditions. Scale bars, 20 μ m.

B) (Top) Representative images of P-S1PR1 immunostaining on hippocampal cells with or without S1P. (Bottom) Plot of the relative fluorescence of P-S1PR1 signals normalized to total S1PR1 signal for control (BSA only) from cells cultured with various concentrations of S1P. ** $P < 0.001$ ($n = 30-40$ cells; one-way ANOVA).

C) Plot of the numbers of branch points and neurite lengths of cells cultured in medium only, BSA only, and with 1 nM, 10 nM, and 100 nM S1P. ** $P < 0.001$ ($n = 60-70$ cells; one-way ANOVA).

D) (Left) Representative images of cultured neurons with either shLuc or shS1PR1 expression for 2 days. (Right) Plots of the lengths and numbers of branch points in cells cultured in DMSO or 10 nM S1P. ** $P < 0.001$ ($n = 50-60$ cells; one-way ANOVA).

	Horizontal	Horizontal	Radial	Radial	Outermost granule cell layer (Radial)
	5dpi (8)	7dpi (6)	5dpi (9)	7dpi (11)	Mature
Membrane resistance (ohm)	602 ± 51	589 ± 49	618 ± 64	611 ± 37	247 ± 51
Membrane potential (mV)	-65 ± 8	-67 ± 15	-68 ± 16	-64 ± 11	-71 ± 8
Membrane capacitance (pF)	-10 ± 2	-10 ± 1	-12 ± 3	-11 ± 2	14 ± 3
Action potential with 200ms-50pA injection	Lack	Lack	Lack	Lack	3.8 spikes
Action potential with 200ms-100pA injection	Lack	Lack	Lack	Lack	10.5 spikes

Table S1. Membrane properties of new and mature DGCs (related to Figure 1).

	Horizontal	Horizontal	Radial	Radial
	5dpi (9)	7dpi (6)	5dpi (9)	7dpi (11)
Detected synaptic activity that can be blocked by 5 μ M Bicuculine	0	0	2	4
Lack of synaptic activity	9	6	7	7

Table S2. GABAergic responses of horizontal and radial DGCs (related to Figure 1).

Supplementary Table 3. Membrane properties of 7dpi new DGCs

	shLuc	shS1PR1
Membrane resistance (ohm)	523 ± 45	541 ± 37
Membrane potential (mV)	-67 ± 7	-63 ± 11

Table S3. Membrane properties of 7dpi new DGCs (related to Figure 3).