

Supplemental Information

**Adult neural stem cell activation in mice
is regulated by the day/night cycle
and intracellular calcium dynamics**

Archana Gengatharan, Sarah Malvaut, Alina Marymonchyk, Majid Ghareghani, Marina Snapyan, Judith Fischer-Sternjak, Jovica Ninkovic, Magdalena Götz, and Armen Saghatelian

Supplementary Table 1, related to Figures 1, 2, 4, 5, 6 and 7. Quantification of NSCs proliferation under different conditions.

	Related figure	NSC division length	NSC proliferation (among electroporated cells)	# cells/mice
RFP electroporation in GFAP-GFP mice and Ki67 immunolabeling	Fig. 1D		7.1±1.7%	139/6 mice
GFAP-GFP + P2-mCherry electroporation and Ki67 immunolabeling	Fig. 1E		7.7±1.0%	178/4mice
<i>In vivo</i> division CMV-GFP CMV-RFP + P2-GFP	Fig. 1J Fig 1J	83.3±11.4 min 92.1±20.3 min		20/10 mice 7/3 mice
<i>GFAP-GFP + P2-mCherry electroporation + day/night proliferation</i> Day perfusion (3pm, ZT 8) Night perfusion (3pm, ZT 18)	Fig. 2D		7.9±0.6% 4.1±0.6%	500/4 mice 493/5 mice
<i>Constant light exposure (GFAP-GFP + P2-mCherry electroporation)</i> Control (12h/12h light/dark cycle) Constant dark 3d Constant light (250 lux) 3d Constant dark 7d Constant light (250 lux) 7d	Fig. 2G		7.2±0.6% 5.7±1.1% 17.3±1.1% 2.4±0.6% 24.8±3.5%	860/8 mice 407/6mice 466/6 mice 764/9 mice 530/5 mice
<i>Day/night Luzindole and Melatonin treatment (GFAP-GFP + P2-mCherry electroporation)</i> Control (night) Luzindole (night) Control (day) Melatonin (day) Luzindole (day)	Fig. 2L		6.0±1.0% 15.5±2.3% 6.3±0.4% 3.4±0.2% 6.9±0.6%	263/4 mice 353/4 mice 373/4 mice 442/5 mice 334/4 mice
<i>In vivo</i> division + Luzindole treatment	Fig. 2O	113.6±13.4 min		7/3mice
<i>Luzindole application (GFAP-GFP + P2-mCherry electroporation)</i> DMSO Luzindole 15µM Luzindole 30µM Luzindole 50µM	Fig. 4B		4.1±2.2% 15.1±3.0% 18.5±2.0% 19.9±3.1%	77/3mice 124/4mice 61/3mice 182/4mice

<i>EGF application</i> <i>(GFAP-GFP + P2-mCherry electroporation)</i> BSA EGF	Fig. 5H		5.7±0.6% 13.0±0.9%	75/3 mice 173/4 mice
<i>2APB application</i> DMSO 2APB Thapsigargin	Fig. 6F		2.8±0.9% 9.4±1.8% 11.2±0.8%	179/5 mice 500/5 mice 315 /5 mice
<i>gRNA</i> <i>(GFAP-GFP + gRNA LacZ-P2-Cas9-mCherry</i> <i>or GFAP-GFP + gRNA LacZ-P2-Cas9-mCherry electroporation)</i> LacZ (P10) Itpr2 (P10) LacZ (P60) Itpr2 (P60)	Fig. 6K		6.0±0.2% 15.5±2.4% 7.5±0.6% 13.2±0.6%	359/4 mice 863/5 mice 689/6 mice 847/4 mice
<i>Optogenetic in vivo</i> GFAP-hBACCS2 + gRNA Itpr2-P2-Cas9-mCherry (no stim.) GFAP-GFP + gRNA Itpr2-P2-Cas9-mCherry (opto. stim.) GFAP-hBACCS2+ gRNA Itpr2-P2-Cas9-mCherry (opto. stim.)	Fig. 7E		15.2±1.5% 15.5±1.9% 5.4±1.3%	733/4 mice 567/3 mice 731/4 mice
<i>Optogenetic in vivo + 3d constant light</i> GFAP-GFP + P2-mCherry (opto. stim.) GFAP-hBACCS2 + P2-mCherry (no stim.) GFAP-hBACCS2 + P2-mCherry (opto. stim.)	Fig. 7H		13.6±1.4% 15.4±1.0% 4.7±0.7%	443/4 mice 338/4 mice 265/4 mice

Supplementary Table 2, related to Figures 3 to 6. Quantification of Ca²⁺ dynamics and intracellular levels in NSCs under different conditions

Experiment	Related figure	Ca ²⁺ dynamics						Ca ²⁺ intracellular level (in GFAP-Twitch2B)			
		qNSCs			aNSCs			qNSCs		aNSCs	
		Frequency (mHz)	Amplitude (%)	# cells/ mice	Frequency (mHz)	Amplitude (%)	# cells/ mice	Ratio CFP/YFP (%)	# cells/mice	Ratio CFP/YFP (%)	# cells/mice
GFAP-GCaMP6s <i>in vivo</i>	Fig. 3D	1.6±0.15	148± 27	13/4 mice	0.009±0.004	41±16	7/3 mice				
GFAP-GCaMP6s <i>ex vivo</i>	Fig. 3G	2.8±0.1	146±9.6	126/46 mice	1.0±0.3	60± 24	14/9 mice				
GFAP-GCaMP6s + P2-mcherry <i>ex vivo</i>	Fig. 3J	2.1±0.2	190±26	40/6 mice	0.8±0.2	103± 31	21/4 mice				
GFAP-Twitch2B <i>in vivo</i>	Fig. 3M							30.3±3.8	52/7 mice	100.6 ±18.5	7/3 mice
<i>Luzindole incubation</i>											
Baseline/Control	Fig. 4D	8.8±1.0	185±32	24/7 mice				47.8±2.2	57/ 3 mice	96.0±17.1	6/2 mice
Luzindole	and 4G	5.1±0.9	189±35					105.2±8.4	42/ 4 mice	99.9±13.7	12/4 mice
<i>EGF application</i>											
Baseline	Fig. 5D	2.7±0.2	151±22	28/11 mice	1.1±0.2	68±21	11/8 mice	31.3±3.0	14/ 4 mice	84.7±11.6	4/2 mice
EGF	and 5F	1.7±0.2	91±14		0.5±0.1	64±27		70.7±12.5	23/ 4 mice	102.6±2.1	4/3 mice
<i>2APB application</i>											
Baseline	Fig. 6C	2.5±0.2	129±17	29/16 mice	0.9±0.3	22±13	6/4 mice	34.6±1.9	42/6 mice	106.0±14.4	7/3 mice
2APB	6D and 6E	1.0±0.3	77±20		0.8±0.3	27±9		61.9±6.7	48/5 mice	101.8±10.6	13/4 mice
<i>Thapsigargin application</i>											
Baseline	Fig. 6C	2.7±0.2	157±19	35/14 mice	1.1±0.4	54±20	7/4 mice				
Thapsigargin	and 6D	1.9±0.2	134±14		1.0±0.4	62 ±18					
<i>AP5 application</i>											
Baseline	Fig. 6C	2.7±0.3	139±21	26/7 mice							
AP5	and 6D	2.7±0.3	121±32								
<i>SKF application</i>											
Baseline	Fig. 6C	3.3±0.4	163±22	22/4 mice							
SKF	and 6D	3.2±0.3	142±26								
<i>HC067047 application</i>											
Baseline	Fig. 6C	2.9 ±0.6	142±32	14/5 mice							
HC067047	and 6D	2.0±0.5	98 ±20								
<i>gRNA</i>											
LacZ	Fig. 6I	2.6 ±0.2	116±18	53/11 mice	0.3±0.1	28±20	9/6 mice				
Itpr2		1.6±0.1	148±14	101/19 mice	0.6±0.5	49±32	6/5 mice				