Supplementary Table 1 The total number of spines analyzed at 0 h, the number of newlyformed spines over 0-8 h, eliminated new spines over 8-16 h and newlyformed spines over 16-24 h in each mouse at postnatal day 21 (P21) (Data related to **Fig.1f, 2a-c** and **Supplementary Fig. 5a**).

				ND	(during c	levelopmer	nt)									
	# of	# of new	# of	Rate of	# of	# of new		new s	spines	form	ed ov	er 16-2	24 h an	d locat	ed rela	ative
	spines	spines	eliminated	eliminated new	spines	spines	to el	liminat	ed (E) or p	ersiste	ent (P)	new s	oines fo	ormed	over
	at 0 h	over	new spines	spine over	at 16 h	over			•		()-8 ĥ [°]				
		0-8 h	over	8-16 h (%)		16-24 h	0-2	μm	2-6	μm) µm	10-2	0 µm	> 20	0 µm
			8-16 h				Е	Р	Е	Р	Е	Р	Е	Р	Е	Р
Animal 1	143	14	9	64.29	150	12	4	2	0	1	1	0	2	2	5	7
Animal 2 #																
L cortex	156	14	10	71.43	158	11	5	1	1	0	0	0	0	0	5	10
R cortex	206	17	13	76.47	197	7	2	0	0	0	0	0	1	0	4	7
Animal 3	159	18	11	61.11	153	9	2	0	2	4	1	2	3	1	1	2
Animal 4	257	11	7	63.64	240	16	4	1	0	0	1	1	3	0	8	14
Animal 5	152	11	7	63.64	151	8	0	0	2	1	1	1	0	0	5	6
Animal 6	151	14	9	64.29	150	11	4	0	0	2	1	1	2	3	4	5
Animal 7	153	14	9	64.29	147	8	5	2	1	1	0	0	1	0	1	5
Total	1377	113	75	66.37	1346	82	26	6	6	9	5	5	12	6	33	56
		•	•		•	•			•	•	•				•	
				NREN	Л-d (durin	g developm	nent)									
	# of	# of new	# of	Rate of	# of	# of new	# of	new s	pines	form	ed ov	er 16-2	24 h an	d locat	ed rela	ative
	spines	spines	eliminated	eliminated new	spines	spines	to el	liminat	ed (E) or p	ersiste	ent (P)	new s	oines fo	ormed	over
	at 0 h	over	new spines	spine over	at 16 h	over					()-8 h				
		0-8 h	over	8-16 h (%)		16-24 h	0-2	μm	2-6	μm	6-10) µm	10-2	0 µm	> 20	0 µm
			8-16 h				Е	Р	Е	Р	Е	Р	Е	Р	Е	Р
Animal 1	156	9	5	55.56	151	9	1	0	2	1	0	0	1	0	5	8
Animal 2	260	21	13	61.9	262	17	2	1	1	1	2	1	4	3	8	11
Animal 3	152	17	11	64.71	150	11	3	1	1	2	3	0	1	1	3	7
Animal 4	165	12	8	66.67	164	9	2	1	2	0	1	3	1	0	3	5
Animal 5	137	12	8	66.67	141	9	3	0	0	3	1	0	2	1	3	5
Total	870	71	45	63.38	868	55	11	3	6	7	7	4	9	5	22	36
				REM	ID (during	developme	ent)									
	# of	# of new	# of	Rate of	# of	# of new	# of	new s	spines	form	ed ov	er 16-2	24 h an	d locat	ed rela	ative
	spines	spines	eliminated	eliminated new	spines	spines	to el	liminat	ed (E) or p	ersiste	ent (P)	new s	oines fo	ormed	over
	at 0 h	over	new spines	spine over	at 16 h	over)-8 h				
		0-8 h	over	8-16 h (%)		16-24 h	0-2	μm	2-6	μm) µm	10-2	0 µm	> 20	0 µm
			8-16 h				Е	Р	Е	Р	Е	Р	Е	Р	Е	Р
Animal 1	151	14	7	50	150	8	0	1	1	0	1	2	2	3	4	3
Animal 2 #																
L cortex	167	12	6	50	163	9	2	0	1	1	0	0	0	3	6	5
R cortex	157	13	6	46.15	159	11	2	0	0	1	2	1	1	1	6	8
Animal 3	170	18	5	27.78	169	11	1	2	2	1	0	0	3	2	5	6
7 ti iii ii ai O		9	5	55.56	171	13	0	0	1	1	0	0	1	1	11	11
Animal 4	173	9		33.30												_
	173 151	8	2	25	148	6	0	0	0	0	2	0	0	1	4	5
Animal 4						6 10	0	0	0	0	1	2	0	1 4	4	3
Animal 4 Animal 5	151	8	2	25	148							_				

^{*}In these animals, new spine survival and distribution were quantified from both left (L) and right (R) motor cortices. In figures 1f, each point represents data from one animal.

Supplementary Table 2 The total number of spines analyzed at 0 h, the number of newly-formed spines after FW training over 0-8 h and eliminated new spines over 8-24 h in each mouse at postnatal day 30 (P30) (Data related to **Fig. 1k**).

		NE) (FW running-induced n	new spines over 0-8 h)		
	# of spines	# of new spines	# of eliminated new	Rate of eliminated	# of eliminated	Rate of eliminated new
	at 0 h	over 0-8 h	spines over 8-16 h	new spine over 8-16 h	new spines	spine over 8-24 h (%)
				(%)	over 8-24 h	
Animal 1	153	11	5	45.45	6	54.55
Animal 2	152	7	3	42.86	4	57.14
Animal 3	185	10	6	60	6	60
Animal 4	174	11	6	54.55	7	63.64
Animal 5	160	7	3	42.86	4	57.14
Animal 6	188	13	8	61.54	9	69.23
Total	1012	59	31	52.54	36	61.02
	T ,, , ,			d new spines over 0-8 h)		
	# of spines	# of new spines	# of eliminated new	Rate of eliminated	# of eliminated	Rate of eliminated new
	at 0 h	over 0-8 h	spines over 8-16 h	new spine over 8-16 h	new spines	spine over 8-24 h (%)
				(%)	over 8-24 h	
Animal 1	146	12	6	50	8	66.67
Animal 2	282	19	12	63.16	13	68.42
Animal 3	225	16	9	56.25	10	62.5
Animal 4	222	15	9	60	9	60
Animal 5	150	11	5	45.45	6	54.55
Animal 6	211	14	8	57.14	9	64.29
Total	1236	87	49	56.32	55	63.22
		REN	MD (FW running-induced	I new spines over 0-8 h)		
	# of spines	# of new spines	# of eliminated new	Rate of eliminated	# of eliminated	Rate of eliminated new
	at 0 h	over 0-8 h	spines over 8-16 h	new spine over 8-16 h (%)	new spines over 8-24 h	spine over 8-24 h (%)
Animal 1	145	9	3	33.33	5	55.56
Animal 2	156	8	3	37.5	4	50
Animal 3	153	10	4	40	5	50
Animal 4	153	9	2	22.22	4	44.44
Animal 5	157	12	4	33.33	5	41.67
Animal 6	158	11	4	36.36	6	54.55
Total	922	59	20	33.90	29	49.15

Supplementary Table 3 The total number of spines analyzed at 0 h, the number of newly-formed spines over 0-8 h after BW running, eliminated new spines over 8-16h and newly-formed spines over 16-24 h after FW running in each mouse at postnatal day 30 (P30) (Data related to **Fig. 1m, 2d-f** and **Supplementary Fig. 5b**)

					N	D											
	# of	# of new	# of	Rate of	# of	# of new	# of	new s	spines	form	ed ove	er 16-	24 h an	d locat	ed rela	ative	
	spines	spines	eliminated	eliminated new	spines	spines	to el	iminat	ed (E) or p	ersiste	ent (P)	P) new spines formed over				
	at 0 h	over	new spines	spine over	at 16 h	over)-8 h					
		0-8 h	over	8-16 h (%)		16-24 h	0-2	μm	2-6	μm	6-10) µm	10-2	0 µm	> 20) µm	
			8-16 h				Е	Р	Е	Р	Е	Р	Е	Р	Е	Р	
Animal 1 #																	
L cortex	157	18	12	66.67	168	16	2	0	1	1	3	1	4	1	6	13	
R cortex	153	12	9	75	152	12	3	0	3	1	1	0	2	3	3	8	
Animal 2	195	15	10	66.67	192	12	4	1	2	0	3	2	3	3	0	6	
Animal 3	156	10	6	60	159	11	3	1	1	1	2	1	2	2	3	6	
Animal 4	152	9	5	55.56	153	8	4	1	0	0	0	0	1	0	3	7	
Animal 5	155	7	4	57.14	158	7	2	0	0	0	0	0	0	3	5	4	
Animal 6	165	13	8	61.54	172	14	4	0	2	5	0	0	4	2	4	7	
Total	1133	84	54	64.28	1154	80	22	3	9	8	9	4	16	14	24	51	
						M-d											
	# of	# of new	# of	Rate of	# of	# of new							24 h an				
	spines	spines	eliminated	eliminated new	spines	spines	to el	iminat	ed (E) or p			new s	pines to	ormed	over	
	at 0 h	over	new spines	spine over	at 16 h	over	0.0		0.0)-8 h	10.0		-		
		0-8 h	over 8-16 h	8-16 h (%)		16-24 h		μm	2-6) µm		0 µm	> 20		
A ! 1 4	450	0		00.5	404	_	E	P	E	P	E	P	E	P	E	P	
Animal 1	159	8	5	62.5	164	9	3	0	1	0	1	0	0	3	4	6	
Animal 2	154	12	8	66.67	152	9	4	0	1	0	0	1	1	1	3	7	
Animal 3	152	10	6 7	60	156	9	2	0	1	1	1	1	1	0	4	7	
Animal 4	157	11 14	8	63.64 57.14	160	11 11	5 5	2	1	3	0	2	3	1	2	5	
Animal 5 Animal 6	152 159	15	7	46.67	156 169	13	3	0	4	2	2	1	0	3	4	5 7	
Total	933	70	41	58.57	957	62	22	3	9	8	8	5	6	9	17	37	
Total	933	70	41	30.37	931	02	22	3	9	0	0	3	0	9	17	31	
					RE	MD											
	# of	# of new	# of	Rate of	# of	# of new	# of	new s	spines	form	ed ove	er 16-	24 h an	d locat	ed rela	ative	
	spines	spines	eliminated	eliminated new	spines	spines	to el	iminat	ed (E) or p	ersiste	ent (P)	new s	pines fo	ormed	over	
	at 0 h	over	new spines	spine over	at 16 h	over					C)-8 h					
		0-8 h	over	8-16 h (%)		16-24 h	0-2	μm	2-6	μm	6-10) µm	10-2	0 µm	> 20) µm	
			8-16 h				Е	Р	Е	Р	Е	Р	Е	P	Е	Р	
Animal 1 #																	
L cortex	198	13	5	38.46	204	11	3	1	2	1	1	0	0	2	5	7	
R cortex	296	17	9	52.94	306	14	1	0	1	1	2	1	3	2	7	10	
Animal 2	164	11	4	36.36	169	6	0	0	0	1	1	1	1	2	4	2	
Animal 3	179	16	9	56.25	188	8	2	0	1	2	1	2	2	0	2	4	
Animal 4 #	465	•		0= -	465	_	,	_	_	_		_	_		_	_	
L cortex	162	8	3	37.5	162	6	1	0	0	0	0	0	0	0	5	6	
R cortex	180	17	8	47.06	181	11	0	0	4	3	1	1	2	3	4	4	
Animal 5	151	11	5	45.45	152	6	1	1	0	0	0	0	1	1	4	4	
Animal 6 #	444	0		27.5	4.40	_ ,		١,								١,	
L cortex	141 202	8 19	3 11	37.5 57.90	149 201	4 11	1 2	0	0 2	0	1 2	1	0	0	2 5	3 9	
R cortex Total	1673	120	57	57.89 47.5	1712	77	11	2	10	8	9	7	9	11	38	49	
IUlai	10/3	120	37	47.0	1712	_ ,,	11		10	0	J	_ ′	9	11	30	49	

^{*}In these animals, new spine survival and distribution were quantified from both left (L) and right (R) motor cortices. In Extended Data Fig. 5b, each point represents data from one animal.

Supplementary Table 4 The total number of spines analyzed at 0 h, the number of newlyformed spines over 0-24 h after FW running and eliminated new spines over 24-36 h in each mouse at postnatal day 30 (P30) (Data related **Supplementary Fig. 4c**).

	ND (I	FW running-induced	d new spines over 0-24 h	1)
	# of spines	# of new spines	# of eliminated new	Rate of eliminated new
	at 0 h	over 0-24 h	spines over 24-36 h	spine over 24-36 h (%)
Animal 1	113	10	5	50
Animal 2	155	12	7	58.33
Animal 3	167	14	7	50
Animal 4	163	14	8	57.14
Animal 5	156	13	7	53.85
Animal 6	151	13	6	46.15
Animal 7	165	15	8	53.33
Animal 8	163	17	10	58.82
Animal 9	145	11	6	54.55
Total	1378	119	64	53.78
	REMD	(FW running-induc	ced new spines over 0-8	h)
	# of spines	# of new spines	# of eliminated new	Rate of eliminated new
	at 0 h	over 0-8 h	spines over 8-16 h	spine over 8-16 h (%)
Animal 1	158	13	4	30.77
Animal 2	155	12	3	25
Animal 3	161	15	4	26.67
Animal 4	149	13	4	30.77
Animal 5	136	13	5	38.46
Animal 6	171	14	5	35.71
Total	930	80	25	31.25

Supplementary Table 5 The performance (r.p.m.) of FW running at 16 h and 24 h, and performance improvement (%) of FW running over 16-24 h under various conditions (Data related to **Fig. 2I**).

	N	ID	
	FW performance at 16 h (r.p.m.) Average of 40 trials	FW performance at 24 h (r.p.m.) Average of 20 trials	Performance improvement (%)
Animal 1	35.1	47.5	35.33
Animal 2	26.1	38.81	48.72
Animal 3	34.93	49	40.3
Animal 4	27.81	40.57	45.89
Animal 5	31.05	45.52	46.61
Animal 6	34.35	67.85	97.53
Animal 7	26.45	38.8	46.69
Average	30.83	46.86	51.58
	NRI FW performance at 16 h (r.p.m.)	EM-d FW performance at 24 h (r.p.m.)	Desformance improvement (0/)
	Average of 40 trials	Average of 20 trials	Performance improvement (%)
Animal 1	38.88	53.4	37.36
Animal 2	26.13	35.67	36.52
Animal 3	36.88	59.75	62.03
Animal 4	34.5	60.7	75.94
Animal 5	37.83	57.85	52.94
Animal 6	27.2	46.5	70.96
Animal 7	32.93	48.45	47.15
Average	33.48	51.76	54.7
	RF	MD	
	FW performance at 16 h (r.p.m.)	FW performance at 24 h (r.p.m.)	Performance improvement (%)
	Average of 40 trials	Average of 20 trials	r enormance improvement (70)
Animal 1	24.48	33.95	38.72
Animal 2	34.73	38.33	10.39
Animal 3	26.43	30.65	15.97
Animal 4	25.14	32.05	27.46
Animal 5	42.15	54.1	28.35
Animal 6	31.35	35.95	14.67
Average	30.71	37.51	22.6

Supplementary Table 6 The performance (r.p.m.) of FW running at 0 h and 24 h, and performance improvement (%) of FW running over 0-24 h under various conditions (Data related to **Fig. 3k**).

	1	ND .	
	FW performance at 0 h (r.p.m.) Average of 40 trials	FW performance at 24 h (r.p.m.) Average of 20 trials	Performance improvement (%)
Animal 1	23	34.3	49.13
Animal 2	18.1	36.85	103.65
Animal 3	23.64	41.8	76.8
Animal 4	26.67	46.65	74.94
Animal 5	26.29	44.35	68.69
Animal 6	24.68	41.55	68.33
Average	23.73	40.92	73.59
		EM-d	T
	FW performance at 0 h (r.p.m.)	FW performance at 24 h (r.p.m.)	Performance improvement (%)
	Average of 40 trials	Average of 20 trials	
Animal 1	26.9	47.9	78.04
Animal 2	23.86	37.45	56.98
Animal 3	31.49	53.7	70.54
Animal 4	24.37	42.58	74.75
Animal 5	25.41	46.2	81.79
Animal 6	23.71	42.15	77.79
Average	25.96	45	73.31
	RE	EMD	
	FW performance at 0 h (r.p.m.)	FW performance at 24 h (r.p.m.)	Performance improvement (%)
	Average of 40 trials	Average of 20 trials	
Animal 1	25.98	48.15	85.36
Animal 2	19.06	27.95	46.68
Animal 3	29.67	38.7	30.45
Animal 4	24.35	40.29	65.47
Animal 5	36.12	50.8	40.63
Animal 6	30.37	35.55	17.07
Animal 7	29.12	33.2	14.00
Average	27.81	39.23	42.81