

Table 01. Experimental parameters and counting results from the optical fractionator⁽¹⁾: Reactive astrocytes, and activated microglia counted markers (ΣQ^-) of brain regions of interest of albino Swiss mice adult females at 15^a and 18^a weeks post Injection of infected (ME7) or normal brain homogenates (NBH)⁽¹⁾

<i>Regions of interest and subjects</i>	<i>a(frame) (μm^2)</i>	<i>A(x,y step) (μm^2)</i>	<i>ASF</i>	<i>Average TSF</i>	<i>ssf</i>	<i>Average counting frames</i>	<i>No of sections</i>	<i>Average ΣQ^-</i>
ASTROCYTES								
Septum								
NBH ⁽²⁾	40 x 40	120 x 120	0.11	0.56 ± 0.06	1/5	117.25 ± 17.13	3	98.75 ± 48.92
ME7-15w ⁽²⁾	40 x 40	120 x 120	0.11	0.58 ± 0.14	1/5	105 ± 22.3	3	141.75 ± 15.22
ME7-18w ⁽²⁾	40 x 40	120 x 120	0.11	0.56 ± 0.05	1/5	128.5 ± 15.95	3	206.25 ± 13.5
Polymorphic layer of dentate gyrus								
NBH ⁽²⁾	30 x 30	60 x 60	0.25	0.34 ± 0.04	1/5	185.25 ± 1.89	6	178.5 ± 41
ME7-15w ⁽²⁾	30 x 30	60 x 60	0.25	0.41 ± 0.17	1/5	200 ± 22.6	6	260 ± 45.1
ME7-18w	30 x 30	60 x 60	0.25	0.32 ± 0.05	1/5	231.25 ± 18.39	6	253.75 ± 40.80
CA1								
NBH ⁽²⁾	60 x 60	120 x 120	0.25	0.57 ± 0.04	1/5	184.25 ± 8.01	6	346.25 ± 76.8
ME7-15w ⁽²⁾	60 x 60	120 x 120	0.25	0.59 ± 0.09	1/5	184 ± 2.64	6	317.7 ± 46.2
ME7-18w ⁽²⁾	60 x 60	120 x 120	0.25	0.61 ± 0.05	1/5	190 ± 9.1	6	480.7 ± 65.4
MICROGLIA								
Septum								
ME7-15w ⁽²⁾	40 x 40	120 x 120	0,11	0.70 ± 0.13	1/5	112.75 ± 10.14	3	164.5 ± 41.25
ME7-18w ⁽²⁾	40 x 40	120 x 120	0,11	0.69 ± 0.04	1/5	110.7 ± 7.93	3	356.5 ± 87.1
Polymorphic Layer of Dentate Gyrus								
ME7-15w ⁽²⁾	40 x 40	80 x 80	0,25	0.80 ± 0.14	1/5	146.5 ± 31.07	6	209.75 ± 45.83
ME7-18w ⁽²⁾	45 x 45	80 x 80	0,32	0.90 ± 0.03	1/5	199.5 ± 9.68	6	483.25 ± 122.72
CA1								
ME7-15w ⁽²⁾	60 x 60	120 x 120	0,25	0.75 ± 0.14	1/5	182 ± 2.94	6	344.7 ± 106.31
ME7-18w ⁽²⁾	60 x 60	120 x 120	0,25	0.84 ± 0.08	1/5	195 ± 17.28	6	419.25 ± 51.3

⁽¹⁾Area of the optical dissector counting frame, a(frame); x and y step sizes, A(x,y step); asf, area sampling fraction [a(frame)/A(x,y step)]; tsf, thickness sampling fraction, calculated by the height of optical dissector divided by section thickness, h/section thickness. ssf, section sampling fraction.

⁽²⁾ All evaluations were performed with a 60X objective lens (N.A. 1.4; D.F. 0.75 μm)

Table. 02. Experimental parameters and counting results from the optical fractionator⁽¹⁾: perineuronal nets and neurons counted markers (ΣQ^-) of brain regions of interest of adult females of albino Swiss mice at 15^a (G2) or 18^a (G3) weeks post injection of infected (ME7) or normal brain homogenates (NBH)⁽¹⁾

Regions of Interest and subjects	a(frame) (μm^2)	A(x,y step) (μm^2)	asf	Average TSF	SSF	Average Counting frames	No of Sections	ΣQ^-
PERINEURONAL NETS								
Septum								
ME7-15w ⁽²⁾	70 x 70	120 x 120	0,34	0.89 ± 0.04	1/5	123.25 ± 12.04	3	172.5 ± 43.12
ME7-18w ⁽²⁾	70 x 70	120 x 120	0,34	0.91 ± 0.02	1/5	128.3 ± 24.6	3	134.5 ± 55.5
NBH ⁽²⁾	70 x 70	120 x 120	0,34	0.87 ± 0.02	1/5	165.7 ± 16.88	3	308.7 ± 51.2
CA1								
ME7-15w ⁽²⁾	120 x 120	120 x 120	1.00	0.88 ± 0.02	1/5	171.5 ± 7.42	6	314.5 ± 21.9
ME7-18w ⁽²⁾	120 x 120	120 x 120	1.00	0.88 ± 0.02	1/5	170.7 ± 6.55	6	239 ± 66.23
NBH ⁽²⁾	120 x 120	120 x 120	1.00	0.94 ± 0.02	1/5	237.5 ± 39.34	6	473.75 ± 53.57
NEURONS								
Polymorphic Layer of Dentate Gyrus								
NBH ⁽²⁾	40 x 40	50 x 50	0.64	0.36 ± 0.029	1/5	286 ± 18.1	6	974.7 ± 71.91
ME7-18w ⁽²⁾	40 x 40	50 x 50	0.64	0.35 ± 0.060	1/5	327 ± 16.0	6	1096 ± 118.0
Septum								
NBH ⁽²⁾	30 x 30	100 x 100	0,09	0.39 ± 0.040	1/5	206.2 ± 18.16	3	609.5 ± 13.0
ME7-18w	30 x 30	100 x 100	0,09	0.42 ± 0.005	1/5	203.25 ± 12.36	3	673.5 ± 55.6

⁽¹⁾Area of the optical dissector counting frame, a(frame); x and y step sizes, A(x,y step); asf, area sampling fraction [a(frame)/A(x,y step)]; tsf, thickness sampling fraction, calculated by the height of optical dissector divided by section thickness, h/section thickness. ssf, section sampling fraction.

⁽²⁾ All evaluations were performed with a 60X objective lens (N.A. 1.4; D.F. 0,75 μm)

Table 3. Estimated individual unilateral activated microglial numbers (n) with coefficient of error (CE) of Septum, CA1 and polymorphic layer of dentate gyrus of albino Swiss mice adult females after 15 or 18 weeks post-inoculation of ME7 infected (ME7) or normal brain homogenates (NBH): mean group numbers (Mean, N), standard deviation (S.D.) and individual and mean CEs.

ME7-3/18w	14.89 ± 0.49	11228	0.05	ME7-1/18w	14.62 ± 0.35	9794	0.05	ME7-3/18w	19.14 ± 0.91	24512	0.07
ME7-6/18w	15.33 ± 0.66	9130	0.06	ME7-7/18w	17.15 ± 0.83	10270	0.06	ME7-6/18w	19.54 ± 0.75	29596	0.07
ME7-9/18w	14.2 ± 0.38	8474	0.05	ME7-9/18w	16.88 ± 0.42	12443	0.05	ME7-9/18w	22.0 ± 2.51	16797	0.07
ME7-10/18w	14.13 ± 0.27	5639	0.07	ME7-10/18w	20.34 ± 0.35	11752	0.06	ME7-10/18w	17.84 ± 0.35	22715	0.07
Mean ME7/18w	14.64 ± 0.29	8618	0.06	Mean	17.25 ± 1.18	11065	0.055	Mean	19.63 ± 0.87	23405	0.07
S.D.		± 2307		S.D.		± 1241		S.D.		± 5282	
CV ² =(S.D./Mean) ²		0.073		CV ² =		0.0125		CV ² =		0.050	
CE ²		0.004		CE ²		0.0030		CE ²		0.005	
CE ² /CV ²		0.055		CE ² /CV ²		0.242		CE ² /CV ²		0.10	
CVB ²		0.069		CVB ²		0.0095		CVB ²		0.045	
CVB ² (% of CV ²)		94.52%		CVB ²		76%		CVB ²		90%	
CE ² /CV ²		0.436		CE ² /CV ²		0.0213		CE ² /CV ²		0.05	
CVB ²		0.021		CVB ²		0.0181		CVB ²		0.121	
CVB ² (% of CV ²)		56.76%		CVB ² (% of CV ²)		78.7%		CVB ² (% of CV ²)		95.3%	
ME7-1/15w	14.01 ± 0.13	5599	0.07	ME7-1/15w	15.91 ± 0.73	5599	0.05	ME7-1/15w	15.2 ± 0.30	10454	0.08
ME7-4/15w	21.7 ± 2.50	5055	0.10	ME7-4/15w	24.6 ± 2.20	9888	0.05	ME7-4/15w	25.2 ± 1.88	9869	0.09
ME7-2/15w	18.7 ± 0.80	6450	0.08	ME7-2/15w	21.42 ± 0.68	8325	0.05	ME7-2/15w	19.54 ± 3.17	9416	0.08
ME7-9/15w	13.89 ± 0.27	4393	0.08	ME7-9/15w	14.35 ± 0.16	10699	0.05	ME7-9/15w	19.0 ± 1.52	12274	0.07
Mean ME7/15w	17.1 ± 1.90	5374	0.08	Mean	19.07 ± 2.39	8628	0.05	Mean	19.7 ± 2.06	10503	0.08
S.D.		± 870		S.D.		2247		S.D.		± 1255	
CV ² =(S.D./Mean) ²		0.026		CV ²		0.0676		CV ²		0.0144	
CE ²		0.0064		CE ²		0.0025		CE ²		0.0064	
CE ² /CV ²		0.25		CE ² /CV ²		0.037		CE ² /CV ²		0.44	
CVB ²		0.0196		CVB ²		0.0651		CVB ²		0.0080	
CVB ² (% of CV ²)		75.4%		CVB ²		96.3%		CVB ²		55.5%	

CVB² = CV² - CE²
(CE, coefficient of error; CV coefficient of variation; CVB, biological coefficient of variation).

Table 4. Estimated individual unilateral reactive astrocyte numbers (n) with coefficient of error (CE) of polymorphic layer of dentate gyrus, CA1 and Septum of albino Swiss mice adult females after 15 or 18 weeks post-inoculation of ME7 infected (ME7) or normal brain homogenates (NBH): mean group numbers (Mean, N), standard deviation (S.D.) and individual and mean CEs.

Astrocytes											
Polymorphic layer of Dentate Gyrus				CA1				Septum			
Subjects	Section Thickness	N	CE	Subjects	Section Thickness	N	CE	Subjects	Section Thickness	N	CE
NBH4	22.05 ± 0.38	12672	0.06	NBH-5	24.5 ± 0.22	12120	0.06	NBH-4	22.2 ± 0.95	8291	0.097
NBH5	22.44 ± 1.32	8147	0.06	NBH-6	23.4 ± 1.2	12092	0.05	NBH-5	26.44 ± 3.82	9552	0.11
NBH-6	27.2 ± 4.10	9946	0.06	NBH-8	23.9 ± 3.7	10458	0.06	NBH-6	26.6 ± 1.2	8833	0.11
NBH-9	23.8 ± 0.30	9041	0.06	NBH-9	21.5 ± 0.9	15229	0.05	NBH-8	21.6 ± 1.14	7157	0.13
NBH Mean	23.87 ± 1.17	9951	0.06	NBH Mean		12475	0.055	NBH Mean	24.21 ± 1.34	8458	0.11
S.D.		1956		S.D.		± 1994		S.D.		± 1010	
CV ² = (S.D./Mean) ²		0.038		CV ²		0.0255		CV ² =		0.0142	
CE ²		0.0036		CE ²		0.003		CE ²		0.0121	
CE ² /CV ²		0.095		CE ² /CV ²		0.119		CE ² /CV ²		0.85	
CVB ²		0.0344		CVB ²		0.0225		CVB ²		0.0021	
CVB ² (% of CV ²)		90.5%		CVB ²		88.2%		CVB ²		14.8%	
ME7-4/15w ⁽²⁾	25.9 ± 1.81	15778	0.07	ME7-2/15w	25.66 ± 0.79	12399	0.058	ME7-3/15w	25.54 ± 0.85	10569	0.12
ME7-2/15w ⁽²⁾	23.95 ± 1.04	15345	0.05	ME7-4/15w	27.19 ± 0.87	13921	0.054	ME7-115w	24.15 ± 2.17	12681	0.10
ME7-8/15w ⁽²⁾	25.40 ± 1.41	14936	0.05	ME7-8/15w	21.03 ± 0.27	8617	0.052	ME7-215w	27.93 ± 1.55	13768	0.10
ME7-9/15w ⁽²⁾	12.54 ± 0.55	10260	0.06	ME7-9/15w	19.79 ± 0.57	10699	0.059	ME7-915w	16.61 ± 0.46	8669	0.12
ME7/15w Mean	21.95 ± 3.16	14080	0.057	ME7/15w Mean	19.07 ± 2.39	11409	0.056	ME7/15w Mean		11422	0.11
S.D.		± 2570		S.D.		2280		S.D.		2265	
CV ² = (S.D./Mean) ²		0.0324		CV ²		0.0399		CV ²		0.04	
CE ²		0.0032		CE ²		0.0031		CE ²		0.01	
CE ² /CV ²		0.099		CE ² /CV ²		0.079		CE ² /CV ²		0.25	
CVB ²		0.0292		CVB ²		0.0368		CVB ²		0.03	
CVB ² (% of CV ²)		90.12%		CVB ²		92.23%		CVB ²		75%	
ME7-1/18w ⁽²⁾	20.0 ± 0.35	13046	0.05	ME7-1/18w	19.33 ± 1.17	13756	0.043	ME7-1/18w	25.5 ± 0.8	15227	0.06
ME7-5/18w ⁽²⁾	24.73 ± 0.22	19046	0.05	ME7-5/18w	22.54 ± 0.24	19752	0.040	ME7-5/18w	23.1 ± 1.2	18036	0.05
ME7-6/18w ⁽²⁾	29.7 ± 2.31	15470	0.05	ME7-6/18w	22.09 ± 0.53	14119	0.042	ME7-6/18w	26.26 ± 1.81	17038	0.05
ME7-7/18w ⁽²⁾	25.8 ± 0.33	14975	0.05	ME7-7/18w	22.90 ± 0.66	16943	0.040	ME7-7/18w	25.05 ± 0.14	17819	0.07
ME7/18w Mean	14.64 ± 0.29	15634	0.05	Mean	21.51 ± 0.73	16142	0.041	Mean	23.96 ± 1.07	17030	0.0675
S.D.		± 2503		S.D.		± 2796		S.D.		± 1276	
CV ² = (S.D./Mean) ²		0.026		CV ² =		0.0289		CV ² =		0.0056	
CE ²		0.0025		CE ²		0.0017		CE ²		0.0045	
CE ² /CV ²		0.096		CE ² /CV ²		0.0582		CE ² /CV ²		0.8	
CVB ²		0.0235		CVB ²		0.0272		CVB ²		0.0011	
CVB ² (% of CV ²)		90.38%		CVB ²		94.11%		CVB ²		19.7%	

$CVB^2 = CV^2 - CE^2$ (CE, coefficient of error; CV coefficient of variation; CVB, biological coefficient of variation).

Table 05. Estimated Individual Unilateral Perineuronal Nets Numbers (N) with Coefficient of Error (CE) of the Septal Region and CA1 of Albino Swiss Mice Adult Females after 15 or 18 Weeks Post-Inoculation: Mean Group Numbers (Mean, N), Standard Deviation (SD) and Individual and Mean CEs.

<i>Subjects</i>	<i>Section Thickness</i>	<i>N</i>	<i>CE</i>	<i>Subjects</i>	<i>Section Thickness</i>	<i>N</i>	<i>CE</i>
Septum				CA1			
NBH-1	23.2 ± 0.08	5931	0.08	NBH-1	22.01 ± 1.6	2908	0.05
NBH-2	22.7 ± 0.27	5939	0.06	NBH-2	22.15 ± 1.1	2336	0.07
NBH-5	23.6 ± 0.27	4852	0.08	NBH-3	22.12 ± 0.49	2579	0.05
NBH-3	22.5 ± 0.07	4255	0.07	NBH-6	22.09 ± 0.63	2906	0.05
Mean		5244	0.07			2682	0.055
S.D.		± 834				± 277	
CV ² =(S.D. ₂ /Mean)		0.026				0.011	
CE ²		0.005				0.003	
CE ² /CV ²		0.2				0.275	
CVB ²		0.021				0.008	
CVB ² (% of CV ²)		80.77%				72.7%	
ME7-6/15w	21.5 ± 0.06	2055	0.07	ME7-1/15w	22.39 ± 0.09	1773	0.07
ME7-8/15w	22.3 ± 0.26	2386	0.12	ME7-6/15w	22.63 ± 0.12	1621	0.078
ME7-10/15w	22.62 ± 0.27	3794	0.09	ME78/15w	23.36 ± 0.65	1938	0.059
ME7-11/15w	24.09 ± 0.98	3303	0.09	ME710/15w	22.26 ± 0.15	1769	0.07
Mean	22.18 ± 0.24	2884	0.09	Mean		1775	0.069
S.D.		± 804		S.D.		± 129	
CV ² =(S.D. ₂ /Mean)		0.08		CV ² =(S.D. ₂ /Mean) ²		0.0053	
CE ²		0.008		CE ²		0.0048	
CE ² /CV ²		0.1		CE ² /CV ²		0.9	
CVB ²		0.072		CVB ²		0.0005	
CVB ² (% of CV ²)		90%		CVB ² (% of CV ²)		9.43%	
ME7-4/18w ⁽²⁾	21.94 ± 0.09	1643	0.12	ME7-4/18w	22.6 ± 0.1	1299	0.08
ME7-7/18w ⁽²⁾	22.48 ± 0.58	1357	0.16	ME7-5/18w	22.54 ± 0.1	1890	0.07
ME7-5/18w ⁽²⁾	22.19 ± 0.85	2403	0.10	ME7-6/18w	23.59 ± 0.29	1167	0.08
ME7-6/18w ⁽²⁾	21.0 ± 0.10	4060	0.07	ME7-7/18w	22.9 ± 0.18	1107	0.08
Mean	21.90 ± 0.32	2354	0.11			1365	
S.D.		± 1190				± 358	0.08
CV ² =(S.D. ₂ /Mean)		0.25				0.0676	
CE ²		0.01				0.0064	
CE ² /CV ²		0.04				0.095	
CVB ²		0.24				0.0612	
CVB ² (% of CV ²)		96%				90.5%	

CVB² = CV² – CE² (CE, coefficient of error; CV coefficient of variation; CVB, biological coefficient of variation).

Table 06. Estimated individual unilateral neuronal numbers (n) with coefficient of error (CE) of polymorphic layer of dentate gyrus, and Septum of albino Swiss mice adult females after 18 weeks post-inoculation of ME7 infected (ME7) or normal brain homogenates (NBH): mean group numbers (Mean, N), standard deviation (S.D.) and individual and mean CEs.

Neurons							
Polymorphic layer of Dentate Gyrus				Septum			
Subjects	Section Thickness	N	CE	Subjects	Section Thickness	N	CE
NBH-3	28.29 ± 0.29	24469	0.052	NBH-4	20.88± 0.22	84890	0.044
NBH-8	22.00 ± 0.31	20700	0.040	NBH-3	19.64 ± 0.12	82795	0.05
NBH-4	19.67 ± 0.73	18580	0.044	NBH-10	28.37 ± 1.33	120001	0.046
NBH-5	22.43 ± 0.48	25432	0.040	NBH-5	16.37 ± 0.28	72891	0.045
Mean		22295	0.044	Mean		90144	0.046
S.D.		± 3210		S.D.		20581	
$CV^2=(S.D./Mean)^2$		0.021		CV^2		0.0529	
CE^2		0.0020		CE^2		0.0021	
CE^2/CV^2		0.09		CE^2/CV^2		0.04	
CVB^2		0.02		CVB^2		0.0021	
CVB^2 (% of CV^2)		90.48%		CVB^2		96.03%	
ME7-8G3	23.71 ± 0.39	23101	0.042	ME7-4/18w	19.27 ± 0.4	68217	0.049
ME7-10G3	26.51 ± 1.38	35977	0.047	ME7-8/18w	18.7 ± 0.1	88214	0.049
ME7-4G3	21.43 ± 0.39	17493	0.043	ME7-6/18w	19.57 ± 0.14	103285	0.041
ME7-6G3	22.24 ± 1.32	22911	0.044	ME7-10/18w	19.5 ± 0.79	98418	0.043
Mean	23.47 ± 1.12	24870	0.044	Mean	19.26 ± 0.20	89533	0.0455
S.D.		± 7848		S.D.		± 15337	
$CV^2=(S.D./Mean)^2$		0.0961		CV^2		0.0289	
CE^2		0.002		CE^2		0.0020	
CE^2/CV^2		0.021		CE^2/CV^2		0.0716	
CVB^2		0.0941		CVB^2		0.0269	
CVB^2 (% of CV^2)		98.0%		CVB^2		93.1%	

$CVB^2 = CV^2 - CE^2$ (CE, coefficient of error; CV coefficient of variation; CVB, biological coefficient of variation).

