Table 32. Quantitative analysis of Tbr2-, DCX- or GFAP-immunoreactive cells in the subgranular zone of the dentate gyrus of the male offspring at PND 21 exposed to chlorpyryfos during the 2nd half of gestation and lactation periods in rats.

		Chlorpyryfos in the diet		
	Control	2.8 ppm	14 ppm	70 ppm
PND 21				
No. of offspring examined [a]	10	10	10	10
Tbr2 (+) cell count (/mm)	8.4 ± 2.5 [b]	9.6 ± 3.5	7.1 ± 2.2	5.5 ± 1.9*
DCX (+) cell count (/mm)	207.5 ± 42.4	213.6 ± 32.8	199.1 ± 40.7	196.3 ± 29.5
GFAP(+) cell count (/mm)	1.7 ± 0.5	1.5 ± 0.4	1.5 ± 1.0	1.2 ± 0.6
PND 77				
No. of offspring examined [a]	10	10	10	10
Tbr2 (+) cell count (/mm)	2.3 ± 1.1	2.2 ± 1.2	2.2 ± 0.8	2.2 ± 1.8
DCX (+) cell count (/mm)	11.5 ± 4.2	10.3 ± 5.0	9.5 ± 4.0	9.8 ± 4.2
GFAP(+) cell count (/mm)	4.0 ± 1.7	3.4 ± 2.2	3.1 ± 1.5	1.7 ± 0.9

* Significantly different from the control group by Dunnett's test (*P<0.05).

[a] All identical 10 male offspring from 8 dams (one or two animals per dam) were subjected to immunohistochemical analyses in each group. Statistical analysis was performed using the litter as the experimental unit, and litter mean values were subjected to analysis on two offspring samples from the same dam.

[b] Mean±SD.

Table 33. Quantitative analysis of proliferating cells as detected by nuclear immunoreactivity of PCNA and apoptotic cells as detected by TUNEL method in the subgranular zone of the dentate gyrus of the male offspring at PND 21 and 77 exposed to chlorpyryfos during the 2nd half of gestation and lactation periods in rats.

		Chlorpyryfos in the diet		
	Control	2.8 ppm	14 ppm	70 ppm
PND 21				
No. of offspring examined [a]	10	10	10	10
PCNA (+) cell count (/mm)	4.1 ± 1.0 [b]	4.1 ± 1.0	4.0 ± 1.0	2.9 ± 0.7*
Apoptotic cell count (/mm)	0.47 ± 0.40	0.61 ± 0.36	0.48 ± 0.39	0.82 ± 0.61
PND 77				
No. of offspring examined [a]	10	10	10	10
PCNA (+) cell count (/mm)	1.6 ± 0.5	1.8 ± 1.3	1.6 ± 1.2	1.7 ± 1.1
Apoptotic cell count (/mm)	0.05 ± 0.17	0.12 ± 0.22	0.03 ± 0.10	0.03 ± 0.10

* Significantly different from the control group by Dunnett's test or Steel's test (*P<0.05).

[a] All identical 10 male offspring from 8 dams (one or two animals per dam) were subjected to immunohistochemical analyses in each group. Statistical analysis was performed using the litter as the experimental unit, and litter mean values were subjected to analysis on two offspring samples from the same dam.

[b] Mean±SD.

Table 34. Quantitative analysis of reelin- or NeuN-immunoreactive cells in the hilus of the dentate gyrus of the male offspring at PND 21and 77 exposed to chlorpyryfos during the 2nd half of gestation and lactation periods in rats.

		Chlorpyryfos in the diet		
	Control	2.8 ppm	14 ppm	70 ppm
PND 21				
No. of offspring examined [a]	10	10	10	10
Reelin (+) cell count (/mm^2)	44.5 ± 7.0 [b]	48.2 ± 7.2	46.1 ± 11.0	41.6 ± 10.1
NeuN (+) cell count (/mm^2)	406.5 ± 59.8	381.3 ± 97.6	350.2 ± 63.6	352.6 ± 50.7
PND 77				
No. of offspring examined [a]	10	10	10	10
Reelin (+) cell count (/mm^2)	32.7 ± 16.5	25.9 ± 12.5	29.6 ± 12.8	25.5 ± 9.1
NeuN (+) cell count (/mm^2)	114.8 ± 36.6	95.4 ± 27.0	102.9 ± 26.6	119.7 ± 34.9

No significant difference in any treated group from the control group.

[a] All identical 10 male offspring from 8 dams (one or two animals per dam) were subjected to immunohistochemical analyses in each group. Statistical analysis was performed using the litter as the experimental unit, and litter mean values were subjected to analysis on two offspring samples from the same dam.

[b] Mean±SD.

Table 35. Organ weight of male offspring after maternal exposure to chlorpyryfos during the 2nd half of gestation and lactation periods in mice

			Chlorpyryfos in the diet (ppm)		
		Control	4	20	100
PND 21					
Males					
No. of offspring examined		10	10	10	10
Brain	(g)	0.43 ± 0.01[a]	0.41 ± 0.01	0.43 ± 0.04	0.44 ± 0.02
	(g/100g BW)	3.77 ± 0.27	3.87 ± 0.19	4.55 ± 0.32**	3.79 ± 0.44
Liver	(g)	0.45 ± 0.04	0.41 ± 0.06	0.48 ± 0.09	0.45 ± 0.05
	(g/100g BW)	3.98 ± 0.25	3.91 ± 0.27	4.63 ± 0.54**	3.96 ± 0.12
Kidneys	(g)	0.17 ± 0.01	0.16 ± 0.02	0.21 ± 0.06*	0.18 ± 0.02
	(g/100g BW)	1.53 ± 0.09	1.55 ± 0.11	2.06 ± 0.47**	1.54 ± 0.10
PND 77					
Males					
No. of offspring examined		10	10	10	10
Brain	(g)	0.49 ± 0.02	0.48 ± 0.02	0.49 ± 0.02	0.47 ± 0.02
	(g/100g BW)	1.06 ± 0.06	1.08 ± 0.08	1.07 ± 0.07	1.07 ± 0.11
Liver	(g)	2.15 ± 0.26	2.03 ± 0.19	2.08 ± 0.21	2.06 ± 0.16
	(g/100g BW)	4.69 ± 0.46	4.56 ± 0.33	4.54 ± 0.22	4.66 ± 0.26
Kidneys	(g)	0.62 ± 0.05	0.61 ± 0.07	0.66 ± 0.04	0.57 ± 0.04
	(g/100g BW)	1.34 ± 0.10	1.36 ± 0.16	1.44 ± 0.09	1.30 ± 0.12

*,** Significantly different from the control group by Dunnett's test or Steel's test (*$P<0.05$, **$p<0.01$).
Abbreviations: BW, body weight; PND, postnatal day.
[a] Mean±SD.

Table 36. Cholinesterase (ChE) activity of male offspring and dams exposed to chlorpyryfos during the 2nd half of gestation and lactation periods in mice

		Chlorpyryfos in the diet (ppm)		
	Control	4	20	100
Dams				
No. of dams examined	6	6	6	6
Total blood (IU/L)	4325 ± 173[b]	3081 ± 577**	2734 ± 304**	1826 ± 307**
Plasma (IU/L)	8287 ± 1026	4461 ± 1395**	4167 ± 535**	2266 ± 711**
Forebrain (IU/g tissue)	20.7 ± 1.3	19.1 ± 1.8	20.1 ± 1.7	7.4 ± 0.9**
Offspring				
PND 21				
No. of offspring examined[a]	6	6	6	6
Total blood (IU/L)	3398 ± 277	2428 ± 777**	2239 ± 189**	1377 ± 222**
Plasma (IU/L)	4196 ± 295	2503 ± 678**	2686 ± 309**	939 ± 240**
Forebrain (IU/g tissue)	16.0 ± 1.1	14.1 ± 0.9*	16.0 ± 0.8	11.0 ± 1.9**
PND 77				
No. of offspring examined[a]	6	6	6	6
Total blood (IU/L)	2779 ± 741	3034 ± 509	2689 ± 391	2852 ± 248
Plasma (IU/L)	5091 ± 1614	3590 ± 740*	3719 ± 476*	3719 ± 476*
Forebrain (IU/g tissue)	22.9 ± 1.2	20.0 ± 5.9	23.3 ± 1.6	22.4 ± 1.8

*,** Significantly different from the control group by Dunnett's test or Steel's test (*$P<0.05$, **$P<0.01$).
Abbreviations: ChE, cholinesterase; PND, postnatal day
[a] One male offspring per dam (n = 6/group) was subjected to measurement.
[b] Mean±SD.

Table 37. Serum levels of thyroid-related hormones of male offspring after maternal exposure to chlorpyryfos during the 2nd half of gestation and lactation periods in mice

		Chlorpyryfos in the diet (ppm)		
	Control	4	20	100
PND 21				
No. of dams examined	40 (10)[a]	40 (10)	40 (10)	40 (10)
T_3 (ng/dL)	35.60 ± 4.83[b]	29.80 ± 3.11*	33.40 ± 2.51	33.40 ± 3.51
T_4 (μg/dL)	3.30 ± 0.33	2.46 ± 0.55**	2.88 ± 0.22	2.90 ± 0.26
TSH (ng/mL)	0.03 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
PND 77				
No. of dams examined	40 (10)	40 (10)	40 (10)	40 (10)
T_3 (ng/dL)	64.00 ± 4.90	61.80 ± 4.02	62.60 ± 5.27	65.80 ± 3.03
T_4 (μg/dL)	5.26 ± 0.70	5.74 ± 0.48	5.22 ± 0.38	6.62 ± 0.67**
TSH (ng/mL)	0.02 ± 0.01	0.04 ± 0.01	0.03 ± 0.02	0.04 ± 0.01

*, ** Significantly different from the control group by Dunnett's test or Steel's test (*P<0.05, **P<0.01).
Abbreviations: PND, postnatal day; T3, triiodothyronine; T4, thyroxine; TSH, thyroid-stimulating hormone.
[a] Number in parenthesis represents the numbers of the pooled samples for each 3-4 samples.
[b] Mean±SD.

Table 38. Brain weight of offspring after maternal exposure to nicotine hydrogen tartrate salt during the second half of gestation and lactation periods in rats

			Nicotine hydrogen tartrate salt in drinking water		
		0 ppm (Control)	32 ppm	160 ppm	800 ppm
PND 21					
Males					
No. of offspring examined[a]		18	16	20	24
Brain	(g)	1.50 ± 0.03[a]	1.46 ± 0.04	1.50 ± 0.05	1.44 ± 0.06*
	(g/100g BW)	3.04 ± 0.07	3.42 ± 0.47	3.22 ± 0.18	3.55 ± 0.35**
Females					
No. of offspring examined[a]		18	16	20	24
Brain	(g)	1.44 ± 0.03	1.40 ± 0.04	1.45 ± 0.03	1.40 ± 0.05**
	(g/100g BW)	3.01 ± 0.11	3.45 ± 0.47	3.19 ± 0.21	3.52 ± 0.46**
PND 77					
Males					
No. of offspring examined[a]		18	16	20	24
Brain	(g)	2.08 ± 0.03	2.03 ± 0.06	2.08 ± 0.04	1.99 ± 0.04**
	(g/100g BW)	0.45 ± 0.02	0.47 ± 0.02	0.48 ± 0.02	0.47 ± 0.02
Females					
No. of offspring examined[a]		18	16	20	24
Brain	(g)	1.92 ± 0.05	1.89 ± 0.06	1.92 ± 0.04	1.84 ± 0.05**
	(g/100g BW)	0.68 ± 0.04	0.72 ± 0.03	0.72 ± 0.05	0.70 ± 0.04

*, ** Significantly different from the untreated controls by Dunnett's test or Steel's test (*P<0.05, **P<0.01).
Abbreviations: BW, body weight; PND, postnatal day.
[a] Two offspring of each sex per dam (n = 9 for 0 ppm, 8 for 2 ppm, 10 for 10 ppm, 12 for 50ppm) were subjected to autopsy and organ weight measurement at each time point. Statistical analysis was performed using the litter as the experimental unit, and mean values were estimated as a litter value when two offspring were examined from the same dam.
[b] Mean±SD.

Table 39. Urinary cotinine level on postnatal day 19 of male offspring and dams exposed to nicotine hydrogen tartrate salt during the second half of gestation and lactation periods in rats

		Nicotine hydrogen tartrate salt in drinking water		
	0 ppm (Control)	2 ppm	10 ppm	50 ppm
Dams				
No. of dams examined	5	5	5	5
Cotinine (ng/mL)	0.2 ± 0.3[b]	72.7 ± 26.1*	919.1 ± 487.6*	5833.4 ± 1772.6*
Offspring				
No. of offspring examined[a]	5	5	5	5
Cotinine (ng/mL)	2.5 ± 0.8	41.7 ± 21.3*	233.8 ± 27.8*	1208.7 ± 1527.1*

* Significantly different from the control group by Dunnett's test or Steel's test (*P<0.05).
[a] One male offspring per dam (n = 5/group) was subjected to analysis.
[b] Mean±SD.

Table 40. Real-time PCR analysis in the hippocampus of offspring after maternal exposure to nicotine hydrogen tartrate salt during the 2nd half of gestation and lactation periods.

	Nicotine hydrogen tartrate salt in drinking water			
	0 ppm (Control)	2 ppm	10 ppm	50 ppm
No. of offspring examined [a]	6	6	6	5
Chrna7	0.93 ± 0.26 [b]	1.09 ± 0.36	1.07 ± 0.23	1.04 ± 0.15
Chrnb2	0.99 ± 0.18	1.13 ± 0.28	1.05 ± 0.13	0.91 ± 0.18
Dcx	0.95 ± 0.39	0.96 ± 0.17	1.21 ± 0.14	1.04 ± 0.16
Dpysl3	1.00 ± 0.19	1.00 ± 0.14	1.09 ± 0.18	0.97 ± 0.15
Reln	0.97 ± 0.22	1.07 ± 0.38	1.10 ± 0.21	0.98 ± 0.15
Pcna	1.01 ± 0.19	1.02 ± 0.19	1.06 ± 0.20	0.96 ± 0.09

No statistically significant differences in any treatment group from the untreated controls.
[a] One male offspring per dam (n = 6/group) was subjected to analysis.
[b] Mean±SD.
Abbreviations: *Chrna7,* cholinergic receptor, nicotinic, alpha7; *Chrnb2,* cholinergic receptor, nicotinic, beta 2; *Dcx*, doublecortin; *Dpysl3*, dihydropyrimidinase-like 3; *Reln*, reelin; *Pcna*, proliferating cell nuclear antigen;

Table 41. Detailed immunohistochemical staining results of Reelin, GAD67 and PCNA, as well as data of apoptotic bodies in the acrylamide-exposure study.

Antigen	ACR	Males			Females			Combined		
Reelin	0 ppm	19.50 ± 9.87	(3)	[a]	21.65 ± 7.75	(7)		21.00 ± 7.92	(10)	
	25 ppm	31.29 ± 10.91	(4)		27.54 ± 5.99	(6)		29.04 ± 7.96	(10)	*
	50 ppm	39.80 ± 6.51	(8)	**	33.01 ± 12.02	(6)		36.89 ± 9.52	(14)	**
	100 ppm	33.52 ± 5.61	(5)	*	37.73 ± 7.85	(3)	*	35.10 ± 6.35	(8)	**
	i.p.	33.57 ± 8.43	(4)		34.07 ± 8.02	(5)	*	33.85 ± 7.67	(9)	**
GAD67	0 ppm	20.02 ± 10.26	(3)		33.23 ± 4.75	(7)	#	29.27 ± 8.90	(10)	
	25 ppm	38.17 ± 3.24	(4)		36.04 ± 10.48	(6)		36.90 ± 8.11	(10)	
	50 ppm	44.77 ± 9.96	(8)	**	39.65 ± 8.12	(6)		42.58 ± 9.26	(14)	**
	100 ppm	47.95 ± 7.75	(5)	**	35.68 ± 12.38	(3)		43.35 ± 10.88	(8)	**
	i.p.	41.77 ± 8.54	(4)	*	38.96 ± 11.45	(5)		40.21 ± 9.75	(9)	*
Calb-D-28K	0 ppm	39.80 ± 6.51	(3)		40.39 ± 13.05	(7)		40.21 ± 11.09	(10)	
	25 ppm	62.35 ± 8.72	(4)	*	38.10 ± 17.90	(6)	#	47.80 ± 18.98	(10)	
	50 ppm	49.33 ± 19.26	(8)		40.25 ± 13.53	(6)		45.44 ± 17.08	(14)	
	100 ppm	58.61 ± 21.33	(5)		55.52 ± 14.62	(3)		57.45 ± 17.99	(8)	*
	i.p.	46.40 ± 7.50	(4)		54.18 ± 18.18	(5)		50.72 ± 14.25	(9)	
PCNA	0 ppm	1.06 ± 0.52	(3)		1.54 ± 0.82	(7)		1.39 ± 0.75	(10)	
	25 ppm	1.27 ± 0.49	(4)		1.78 ± 0.58	(6)		1.58 ± 0.58	(10)	
	50 ppm	1.59 ± 0.70	(8)		1.63 ± 0.33	(6)		1.61 ± 0.55	(14)	
	100 ppm	1.44 ± 0.68	(5)		1.09 ± 0.58	(3)		1.31 ± 0.63	(8)	
	i.p.	1.43 ± 0.68	(4)		1.12 ± 0.32	(5)		1.26 ± 0.50	(9)	
Apoptotic bodies	0 ppm	0.67 ± 0.47	(3)		0.61 ± 0.51	(7)		0.63 ± 0.47	(10)	
	25 ppm	0.28 ± 0.07	(4)		0.26 ± 0.32	(5)		0.27 ± 0.23	(9)	
	50 ppm	0.23 ± 0.18	(8)		0.36 ± 0.17	(6)		0.28 ± 0.18	(14)	
	100 ppm	0.22 ± 0.14	(5)		0.11 ± 0.09	(3)	*	0.18 ± 0.13	(8)	*
	i.p.	0.35 ± 0.32	(4)		0.09 ± 0.09	(5)	*	0.21 ± 0.24	(9)	*

[a] Mean ± SD.
*P < 0.05, ** P < 0.01 vs. 0 ppm group.
# P < 0.05 vs. males in the same group.
() No. of the animals examined.

Table 42. Effects on dams and reproductive parameters in Experiment 1 of the acrylamide study

	AA in the drinking water (ppm)			
	0 (control)	4	20	100
No. of dams examined	6	6	6	5
Maternal parameters				
BW gain (g/day)				
GD 10- GD 20	10.05 ± 1.28 [a]	10.12 ± 1.86	8.62 ± 3.49	7.60 ± 3.92
Day 1–Day 10 after delivery	0.22 ± 0.66	1.19 ± 0.59	0.47 ± 1.13	-0.34 ± 0.61
Day 10–Day 21 after delivery	-2.74 ± 1.18	-3.34 ± 1.15	-3.13 ± 1.36	-1.66 ± 0.94
Food intake (g/day)				
GD 10–GD 20	19.72 ± 2.53	18.39 ± 2.01	17.33 ± 3.43	16.47 ± 1.09
Day 1–Day 10 after delivery	26.34 ± 2.54	26.30 ± 0.87	24.53 ± 5.74	22.64 ± 2.15
Day 10–Day 21 after delivery	38.94 ± 2.43	42.06 ± 2.50	38.60 ± 7.97	33.14 ± 3.47
Water consumption (ml/day)				
GD 10–GD 20	33.75 ± 3.94	30.33 ± 4.85	30.85 ± 6.24	28.79 ± 3.82
Day 1–Day 10 after delivery	35.38 ± 2.69	42.58 ± 5.75	36.44 ± 7.58	34.59 ± 5.31
Day 10–Day 21 after delivery	52.05 ± 2.60	55.32 ± 6.23	55.16 ± 14.56	44.13 ± 5.23
AA intake (mg/kg BW/day)				
GD 10–GD 20	0	0.36 ± 0.06	1.77 ± 0.27	8.26 ± 2.16
Day 1–Day 10 after delivery	0	0.51 ± 0.07	2.12 ± 0.37	11.78 ± 1.92
Day 10–Day 21 after delivery	0	0.89 ± 0.08	4.29 ± 0.95	18.74 ± 1.88
Necropsy at day 21 after delivery				
BW (g)	321.0 ± 31.4	312.6 ± 18.4	325.0 ± 35.1	280.4 ± 28.9
Brain weight				
(g)	1.96 ± 0.09	1.98 ± 0.04	1.95 ± 0.03	1.88 ± 0.08
(g/100 g BW)	0.61 ± 0.04	0.63 ± 0.04	0.61 ± 0.07	0.67 ± 0.06
Liver weight				
(g)	14.44 ± 1.78	12.16 ± 0.87	13.15 ± 1.88	10.90 ± 2.06**
(g/100 g BW)	4.50 ± 0.41	3.89 ± 0.22	4.04 ± 0.36	3.89 ± 0.61
Reproductive parameters				
Duration of pregnancy	21.17 ± 0.41	20.83 ± 0.41	21.17 ± 0.41	21.00 ± 0.00
No. of implantation sites	10.33 ± 2.60	12.75 ± 2.16	13.08 ± 3.44	10.33 ± 2.23
No. of live offspring	11.67 ± 3.14	11.83 ± 3.37	10.83 ± 5.78	10.67 ± 5.50
Male ratio (%)	47.2 ± 13.8	47.1 ± 17.8	41.3 ± 21.7	42.3 ± 14.2

** $P < 0.01$ vs. 0 ppm control group (Dunnett's or Steel's test).

[a] Mean ± SD.

Table 43. Body and brain weights of male pups exposed to acrylamide during the 2nd half of gestation and lactation periods in rats.

	Experiment 1				Experiment 2	
	AA in the drinking water (ppm)				Intraperitoneal injections	
	0 (control)	4	20	100	Saline (control)	AA
PND 21						
No. of animals examined	12	11	11	10	12	12
BW (g)	38.05±1.59 [a]	39.26±1.03	39.59±3.39	30.80±2.16**	46.82±1.07	31.56±4.23##
Brain weight						
(g)	1.44±0.04	1.39±0.06	1.41±0.04	1.35±0.03*	1.44±0.03	1.15±0.08##
(g/100 g BW)	3.81±0.09	3.55±0.12	3.58±0.26	4.43±0.22**	3.10±0.10	3.74±0.35##
PND 77						
No. of animals examined	12	12	12	11	12	12
BW (g)	391.5±16.3	383.1±24.8	380.6±23.6	358.2±20.5**	397.5±29.6	310.9±31.6##
Brain weight						
(g)	1.99±0.08	1.98±0.06	1.96±0.06	1.91±0.05**	2.01±0.07	1.67±0.11##
(g/100 g BW)	0.51±0.02	0.52±0.04	0.52±0.03	0.53±0.03	0.51±0.04	0.54±0.03

* $P < 0.05$, ** $P < 0.01$ vs. 0 ppm control group (Dunnett's or Steel's test).

$P < 0.01$ vs. saline control group (Student's or Aspin-Welch's *t*-test).

[a] Mean ± SD.

Table 44. Density of reelin or NeuN-immunoreactive cells in the dentate hilus of male exposed to acrylamide during the 2nd half of gestation and lactation periods in rats

	Experiment 1				Experiment 2	
	AA in the drinking water (ppm)				Intraperitoneal injections	
	0 (control)	4	20	100	Saline (control)	AA
PND 21						
No. of animals examined	12	11	11	10	12	12
reelin (/mm²)	68.0±8.3 [a]	66.6±7.5	75.7±7.1	89.4±13.9**	67.0±8.9	88.5±13.0##
NeuN (/mm²)	158.9±27:3	154.1±15.0	174.8±24.6	223.8±25.5**	149.8±18.6	183.0±41.1#
PND 77						
No. of animals examined	12	12	12	11	12	12
reelin (/mm²)	47.0±5.5	46.5±4.5	49.3±6.6	51.8±5.6	49.2±11.3	55.3±8.1
NeuN (/mm²)	85.0±15.1	81.7±14.1	100.2±13.1*	105.5±13.4**	80.5±13.2	96.9±13.8##

* $P < 0.05$, ** $P < 0.01$ vs. 0 ppm control group (Dunnett's or Steel's test).

# $P < 0.05$, ## $P < 0.01$ vs. saline control group (Student's or Aspin-Welch's *t*-test).

[a] Mean ± SD.

Table 45. Distribution of PCNA-immunoreactive cells and apoptotic bodies in the SGZ of male exposed to acrylamide during the 2nd half of gestation and lactation periods in rats [a]

	Experiment 1				Experiment 2	
	AA in the drinking water (ppm)				Intraperitoneal injections	
	0 (control)	4	20	100	Saline (control)	AA
PND 21						
No. of animals examined	12	11	11	10	12	12
PCNA (/mm)	5.06±1.43 [b]	4.81±0.51	3.37±0.56**	2.90±0.60**	4.67±0.80	3.46±0.74##
Apoptotic bodies (/mm)	0.34±0.27	0.28±0.13	0.16±0.09	0.17±0.10	0.21±0.15	0.14±0.12
PND 77						
No. of animals examined	12	12	12	11	12	12
PCNA (/mm)	0.73±0.24	0.76±0.39	0.69±0.33	0.78±0.25	0.81±0.22	0.71±0.23
Apoptotic bodies (/mm)	0.01±0.01	0.00±0.01	0.01±0.01	0.00±0.01	0.00±0.01	0.01±0.02

** P < 0.01 vs. 0 ppm control group (Dunnett's or Steel's test).
P < 0.01 vs. saline control group (Student's or Aspin-Welch's *t*-test).
[a] Profile counting method was applied.
[b] Mean ± SD.

Table 46. Distribution of TUNEL-positive apoptotic cells in the SGZ of pups at PND 21 after exposure to acrylamide during the 2nd half of gestation and lactation periods in rats [a]

	Experiment 1				Experiment 2	
	AA in the drinking water (ppm)				Intraperitoneal injections	
	0 (control)	4	20	100	Saline (control)	AA
PND 21						
No. of animals examined	12	11	11	10	12	12
TUNEL (/mm)	0.15±0.18 [b]	0.15±0.17	0.05±0.10	0.10±0.16	0.17±0.13	0.09±0.11

[a] Number of positive cells was measured in one section in each brain.
[b] Mean ± SD.

Table 47. Distribution of immunoreactive cells for neuronal stage-defining markers in the SGZ of male pups at PND 21 after exposure to acrylamide during the 2nd half of gestation and lactation periods in rats [a]

	AA in the drinking water (ppm)	
	0 (control)	100
No. of animals examined	12	10
Dpysl3 (/mm)	5.26±4.64 [b]	1.94±1.30*
Dcx (/mm)	106.6±10.9	81.8±11.7**
NeuroD1 (/mm)	47.8±8.4	48.5±6.0
Tbr2 (/mm)	7.24±1.65	7.11±1.76
Pax6 (/mm)	18.3±3.8	16.6±4.2

* P < 0.05, ** P < 0.01 vs. 0 ppm control group (Student's or Aspin-Welch's *t*-test).
[a] Profile counting method was applied.
[b] Mean ± SD.

Table 48. Distribution of immunoreactive cells for neuronal stage-defining markers in the SGZ of pups at PND 77 after exposure to acrylamide during the 2nd half of gestation and lactation periods in rats [a]

	AA in the drinking water (ppm)	
	0 (control)	100
PND 77		
No. of animals examined	12	11
Dpysl3 (/mm)	0.64±0.64 [b]	0.65±0.29
Dcx (/mm)	17.17±2.24	17.12±2.28

[a] Number of immunoreactive cells for each antigen was measured in one section in each brain.
[b] Mean ± SD.

Table 49. Number of reelin-positive cells in the dentate gyrus of female pups at PND 4 after exposure to acrylamide during the 2nd half of gestation and lactation periods in rats [a]

	AA in the drinking water (ppm)			
	0 (control)	4	20	100
PND 4				
No. of animals examined	10	9	10	10
Molecular layer (/mm)	34.24±4.85 [b]	29.54±6.53	35.51±10.22	34.53±8.44
Hilus (/mm)	11.21±2.08	11.52±4.45	10.26±3.10	12.50±4.95

[a] Number of immunoreactive cells for each antigen was measured in one section in each brain.

[b] Mean ± SD.

Table 50. Density of reelin-immunoreactive cells in the hilus and PCNA-immunoreactive cells in the SGZ of dams in Experiment 1 of the acrylamide study [a]

	AA in the drinking water (ppm)	
	0 (control)	100
No. of animals examined	6	5
reelin ($/mm^2$)	52.98±5.86 [b]	49.85±6.28
PCNA (/mm)	0.25±0.13	0.23±0.06

[a] Profile counting method was applied.

[b] Mean ± SD.

Table 51. Mean area of the hilus and mean length of the SGZ of male pups after exposure to acrylamide during the 2nd half of gestation and lactation periods in rats [a]

	AA in the drinking water (ppm)				Intraperitoneal injections	
	0 (control)	4	20	100	Saline (control)	AA
PND 21						
No. of animals	12	11	11	10	12	12
Hilar area (mm^2)	0.566±0.142 [b]	0.569±0.049	0.572±0.041	0.449±0.072**	0.554±0.054	0.483±0.090#
SGZ-length (mm)	3.134±0.773	3.447±0.316*	3.466±0.174*	2.842±0.414	3.074±0.368	2.992±0.507
PND 77						
No. of animals	12	12	12	11	12	12
Hilar area (mm^2)	0.722±0.062	0.728±0.080	0.693±0.075	0.659±0.053	0.621±0.092	0.541±0.081#
SGZ-length (mm)	4.302±0.397	4.416±0.473	4.224±0.587	3.927±0.464	4.279±0.534	4.054±0.679

* $P < 0.05$, ** $P < 0.01$ vs. 0 ppm control group (Dunnett's or Steel's test).

# $P < 0.05$ vs. saline control group (Student's or Aspin-Welch's *t*-test).

[a] The mean values of the hilar area and the length of the SGZ were measured in three different sections with approximately 250 μm interval using serial sections in each brain.

[b] Mean ± SD.

Table 52. Transcript levels measured by real-time RT-PCR in the hippocampus of male pups at PND 21 after exposure to acrylamide during the 2nd half of gestation and lactation periods in rats

	Experiment 1				Experiment 2	
	AA in drinking water (ppm)				Intraperitoneal injections	
	0 (control)	4	20	100	Saline (control)	AA
No. of animals examined	10	8	8	6	12	7
Real-time PCR normalized to *Actb*						
reelin and related molecules						
Reln	1.01±0.17[a]	0.90±0.21	0.87±0.15	0.86±0.15	1.02±0.22	0.84±0.11
Lrp8	1.01±0.14	1.03±0.20	1.02±0.20	1.10±0.20	1.02±0.20	0.89±0.11
Vldlr	1.01±0.11	1.02±0.13	1.03±0.17	1.04±0.13	1.08±0.57	0.82±0.07
Dab1	1.01±0.11	0.98±0.08	0.99±0.13	1.00±0.16	1.00±0.08	1.08±0.21
Neuronal stage-defining marker molecules						
Pax6	1.01±0.18	0.93±0.21	0.94±0.12	0.86±0.10	1.03±0.26	1.09±0.16
Neurod1	1.03±0.25	0.79±0.21	0.89±0.24	0.88±0.17	1.03±0.23	1.32±0.25#
Dcx	1.02±0.20	0.89±0.31	1.00±0.20	1.08±0.39	1.02±0.19	1.03±0.21
Dpysl3	1.02±0.21	0.95±0.23	0.91±0.17	1.08±0.14	1.01±0.14	0.85±0.11#
Cell proliferation marker molecule						
Pcna	1.00±0.11	0.86±0.09*	0.86±0.14*	0.83±0.09**	1.02±0.23	0.93±0.10
Epigenetic event-related enzymes						
Dnmt1	1.02±0.20	0.95±0.12	0.86±0.11	0.92±0.17	1.01±0.16	0.98±0.07
Hdac1	1.01±0.13	0.92±0.14	1.08±0.17	0.87±0.11	1.02±0.22	0.96±0.13
Hdac2	1.01±0.13	0.87±0.09	0.97±0.17	0.99±0.16	1.01±0.13	1.02±0.11
Hdac8	1.03±0.29	0.85±0.15	0.97±0.18	0.89±0.10	1.06±0.28	1.11±0.15
Real-time PCR normalized to *Gapdh*						
reelin and related molecules						
Reln	1.01±0.15	0.92±0.19	0.91±0.10	0.95±0.15	1.01±0.15	0.98±0.12
Lrp8	1.01±0.14	1.05±0.12	1.06±0.09	1.22±0.17*	1.01±0.15	1.04±0.08
Vldlr	1.00±0.09	1.04±0.05	1.08±0.09	1.16±0.14**	1.11±0.70	0.96±0.10
Dab1	1.01±0.17	1.03±0.19	1.05±0.20	1.11±0.18	1.01±0.14	1.27±0.24#
Neuronal stage-defining marker molecules						
Pax6	1.02±0.22	0.96±0.20	0.99±0.13	0.96±0.13	1.04±0.34	1.28±0.19
Neurod1	1.03±0.25	0.83±0.26	0.95±0.30	0.98±0.21	1.03±0.27	1.54±0.26##
Dcx	1.01±0.17	0.91±0.28	1.08±0.34	1.19±0.42	1.01±0.16	1.21±0.26
Dpysl3	1.01±0.17	0.96±0.15	0.95±0.10	1.19±0.14	1.00±0.10	0.97±0.08
Cell proliferation marker molecule						
Pcna	1.01±0.14	0.89±0.07	0.90±0.15	0.92±0.13	1.01±0.16	1.09±0.07
Epigenetic event-related enzymes						
Dnmt1	1.01±0.17	0.96±0.09	0.91±0.10	1.01±0.21	1.01±0.14	1.15±0.13#
Hdac1	1.00±0.07	0.95±0.15	1.13±0.09	0.98±0.19	1.03±0.28	1.12±0.12
Hdac2	1.01±0.11	0.91±0.19	1.02±0.13	1.11±0.25	1.03±0.28	1.12±0.12
Hdac8	1.02±0.27	0.88±0.18	1.02±0.18	1.00±0.16	1.05±0.26	1.30±0.15#

* $P < 0.05$, ** $P < 0.01$ vs. 0 ppm control group (Dunnett's or Steel's test).

[#] $P < 0.05$, [##] $P < 0.01$ vs. saline control group (Student's or Aspin-Welch's *t*-test).

[a] Mean ± SD.

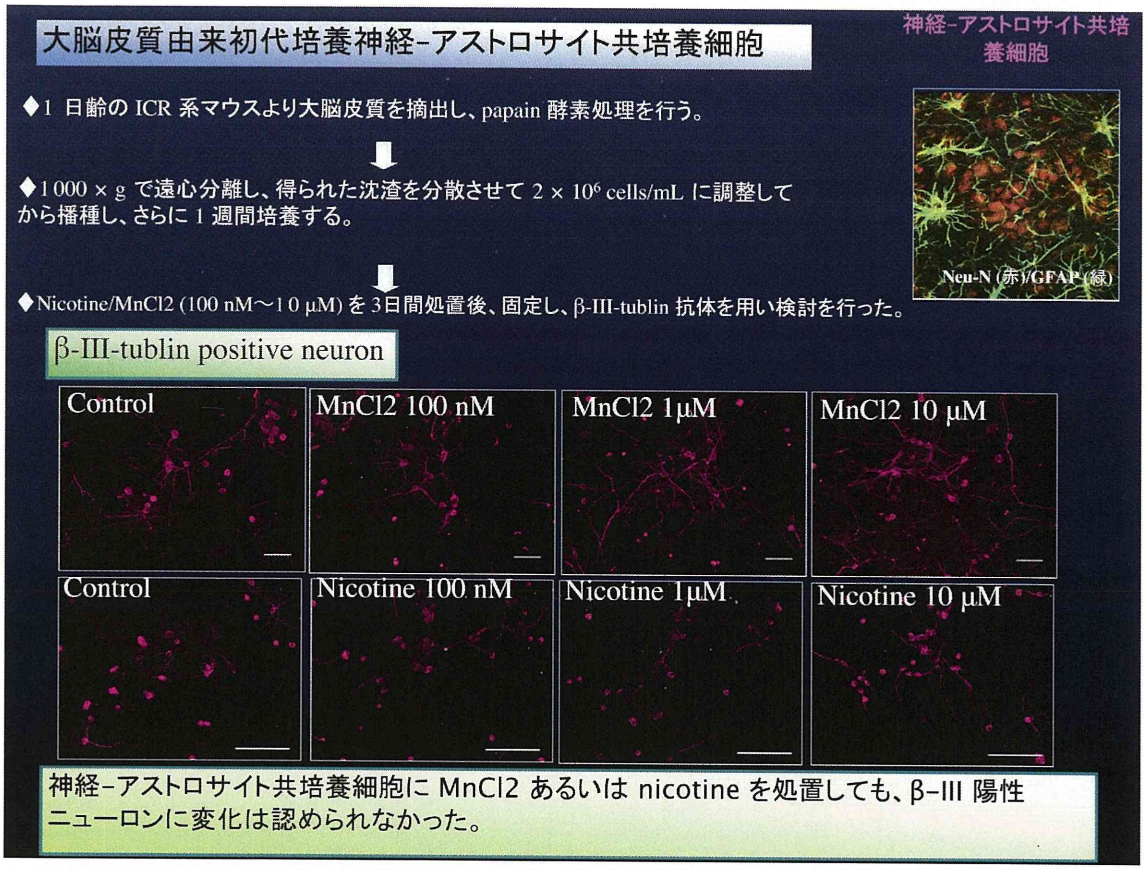

大脳皮質由来初代培養神経-アストロサイト共培養細胞
神経-アストロサイト共培養細胞
◆1 日齢の ICR 系マウスより大脳皮質を摘出し、papain 酵素処理を行う。
◆1000 × g で遠心分離し、得られた沈渣を分散させて 2 × 10^6 cells/mL に調整してから播種し、さらに 1 週間培養する。
◆Nicotine/MnCl2 (100 nM～10 μM) を 3日間処置後、固定し、β-III-tublin 抗体を用い検討を行った。
Neu-N (赤)/GFAP (緑)
β-III-tublin positive neuron
Control
MnCl2 100 nM
MnCl2 1μM
MnCl2 10 μM
Control
Nicotine 100 nM
Nicotine 1μM
Nicotine 10 μM
神経-アストロサイト共培養細胞に MnCl2 あるいは nicotine を処置しても、β-III 陽性ニューロンに変化は認められなかった。

Fig. 47

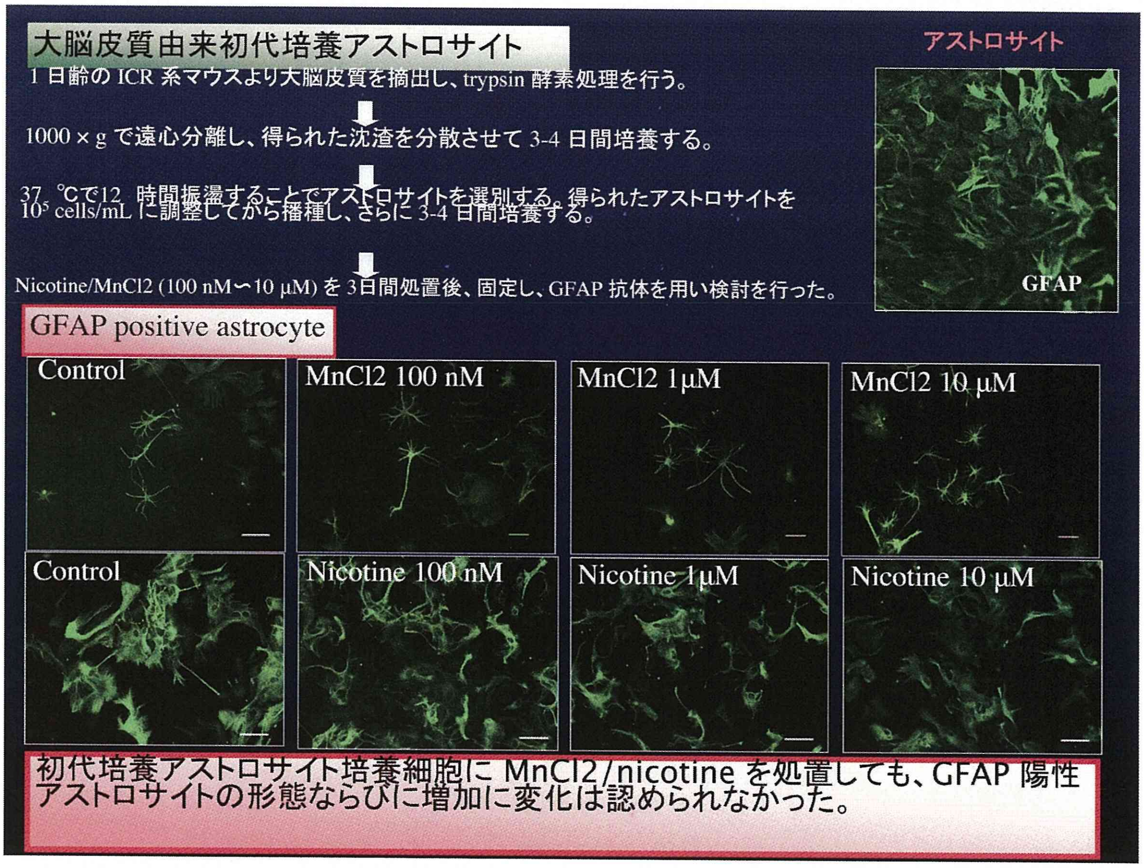

大脳皮質由来初代培養アストロサイト
アストロサイト
1 日齢の ICR 系マウスより大脳皮質を摘出し、trypsin 酵素処理を行う。
1000 × g で遠心分離し、得られた沈渣を分散させて 3-4 日間培養する。
37 ℃で12 時間振盪することでアストロサイトを選別する。得られたアストロサイトを 10^5 cells/mL に調整してから播種し、さらに 3-4 日間培養する。
Nicotine/MnCl2 (100 nM～10 μM) を 3日間処置後、固定し、GFAP 抗体を用い検討を行った。
GFAP
GFAP positive astrocyte
Control
MnCl2 100 nM
MnCl2 1μM
MnCl2 10 μM
Control
Nicotine 100 nM
Nicotine 1μM
Nicotine 10 μM
初代培養アストロサイト培養細胞に MnCl2/nicotine を処置しても、GFAP 陽性アストロサイトの形態ならびに増加に変化は認められなかった。

Fig. 48

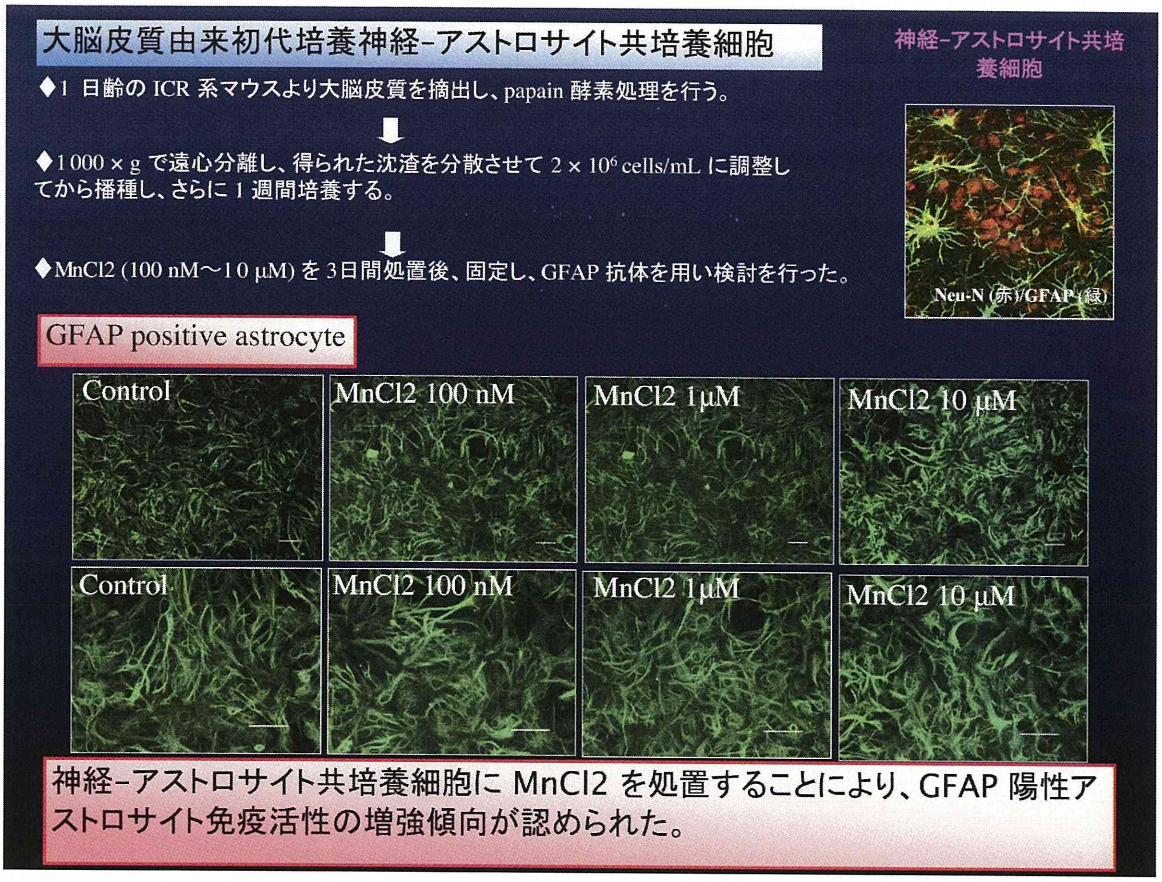

大脳皮質由来初代培養神経-アストロサイト共培養細胞
神経-アストロサイト共培養細胞
◆1 日齢の ICR 系マウスより大脳皮質を摘出し、papain 酵素処理を行う。
◆1 000 × g で遠心分離し、得られた沈渣を分散させて 2 × 10⁶ cells/mL に調整してから播種し、さらに 1 週間培養する。
◆MnCl2 (100 nM～10 μM) を 3日間処置後、固定し、GFAP 抗体を用い検討を行った。
Neu-N (赤)/GFAP (緑)
GFAP positive astrocyte
Control
MnCl2 100 nM
MnCl2 1μM
MnCl2 10 μM
Control
MnCl2 100 nM
MnCl2 1μM
MnCl2 10 μM
神経-アストロサイト共培養細胞に MnCl2 を処置することにより、GFAP 陽性アストロサイト免疫活性の増強傾向が認められた。

Fig. 49

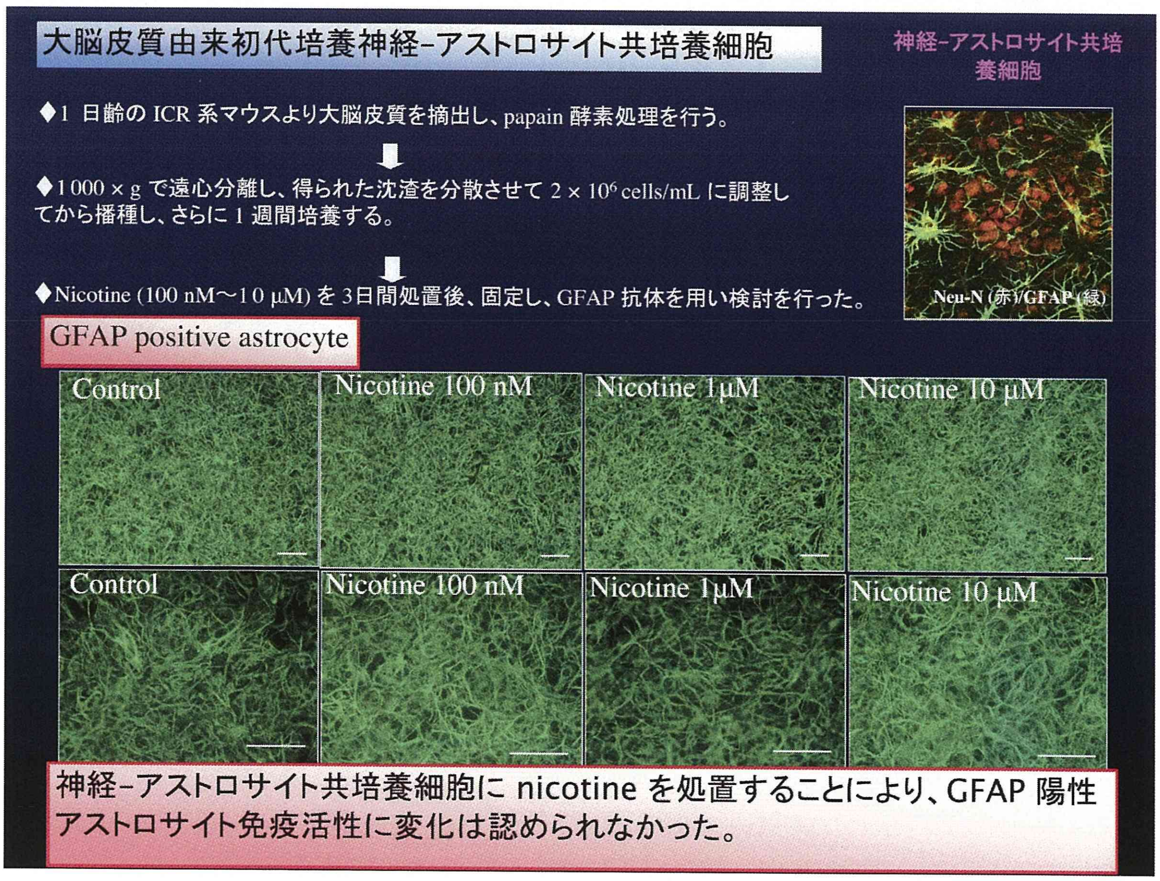

大脳皮質由来初代培養神経-アストロサイト共培養細胞
神経-アストロサイト共培養細胞
◆1 日齢の ICR 系マウスより大脳皮質を摘出し、papain 酵素処理を行う。
◆1 000 × g で遠心分離し、得られた沈渣を分散させて 2 × 10⁶ cells/mL に調整してから播種し、さらに 1 週間培養する。
◆Nicotine (100 nM～10 μM) を 3日間処置後、固定し、GFAP 抗体を用い検討を行った。
Neu-N (赤)/GFAP (緑)
GFAP positive astrocyte
Control
Nicotine 100 nM
Nicotine 1μM
Nicotine 10 μM
Control
Nicotine 100 nM
Nicotine 1μM
Nicotine 10 μM
神経-アストロサイト共培養細胞に nicotine を処置することにより、GFAP 陽性アストロサイト免疫活性に変化は認められなかった。

Fig. 50

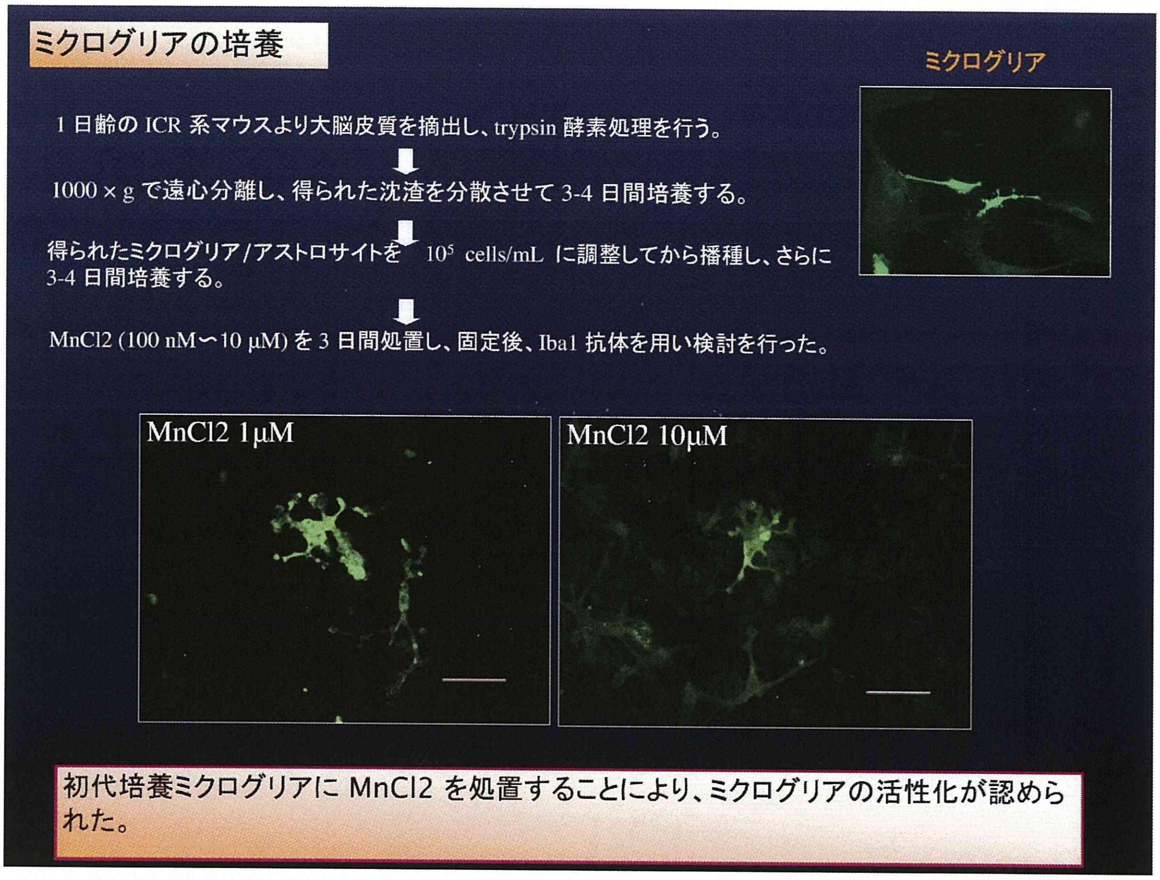

ミクログリアの培養
ミクログリア
1日齢のICR系マウスより大脳皮質を摘出し、trypsin酵素処理を行う。
1000 × g で遠心分離し、得られた沈渣を分散させて3-4日間培養する。
得られたミクログリア/アストロサイトを 10⁵ cells/mL に調整してから播種し、さらに3-4日間培養する。
MnCl2 (100 nM〜10 μM) を3日間処置し、固定後、Iba1抗体を用い検討を行った。
MnCl2 1μM
MnCl2 10μM
初代培養ミクログリアにMnCl2を処置することにより、ミクログリアの活性化が認められた。

Fig. 51

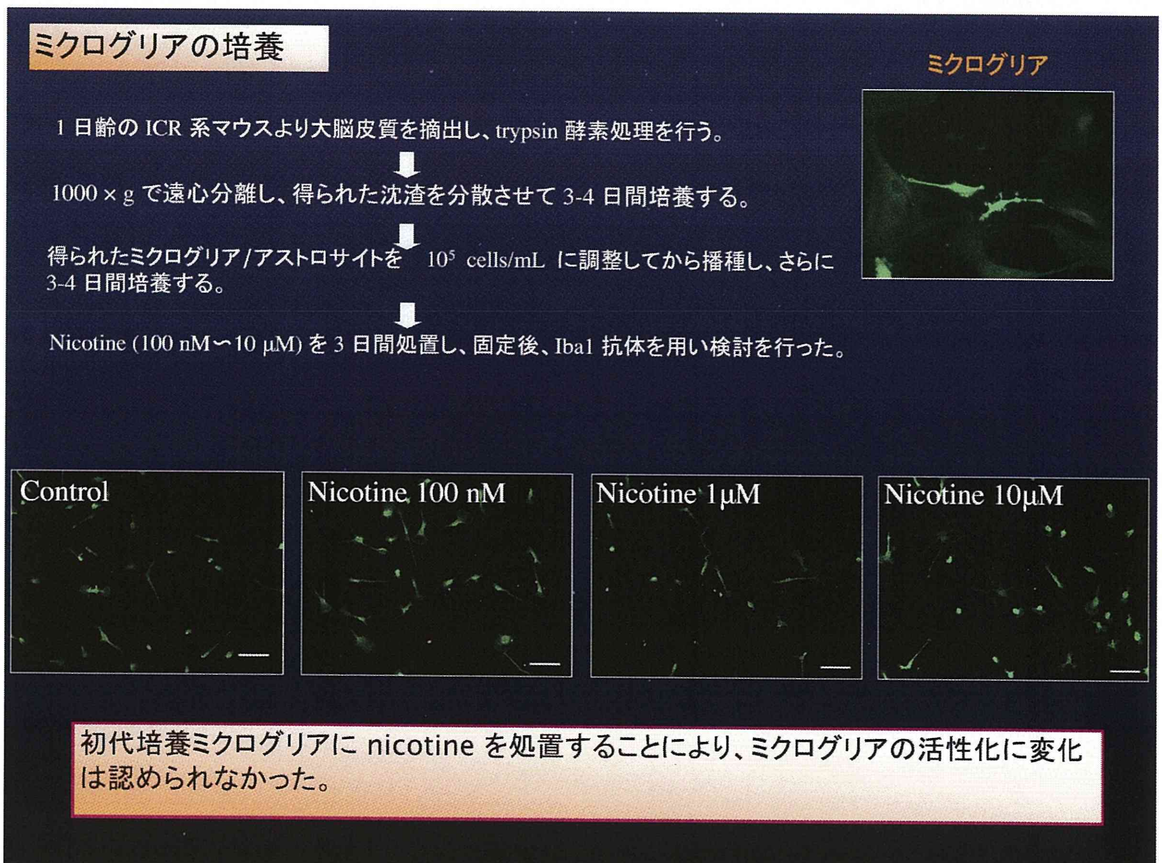

ミクログリアの培養
ミクログリア
1日齢のICR系マウスより大脳皮質を摘出し、trypsin酵素処理を行う。
1000 × g で遠心分離し、得られた沈渣を分散させて3-4日間培養する。
得られたミクログリア/アストロサイトを 10⁵ cells/mL に調整してから播種し、さらに3-4日間培養する。
Nicotine (100 nM〜10 μM) を3日間処置し、固定後、Iba1抗体を用い検討を行った。
Control
Nicotine 100 nM
Nicotine 1μM
Nicotine 10μM
初代培養ミクログリアにnicotineを処置することにより、ミクログリアの活性化に変化は認められなかった。

Fig. 52

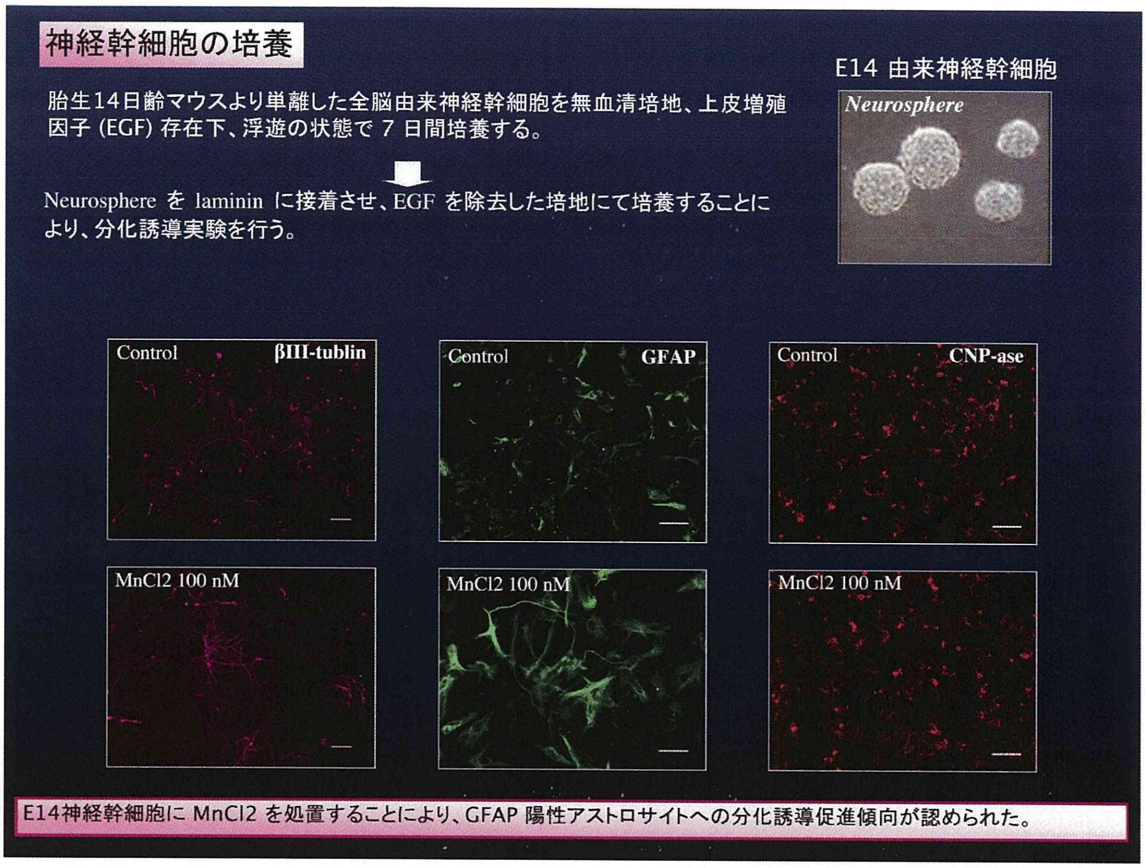

神経幹細胞の培養
E14 由来神経幹細胞
胎生14日齢マウスより単離した全脳由来神経幹細胞を無血清培地、上皮増殖因子 (EGF) 存在下、浮遊の状態で 7 日間培養する。
Neurosphere
Neurosphere を laminin に接着させ、EGF を除去した培地にて培養することにより、分化誘導実験を行う。
Control
βIII-tublin
Control
GFAP
Control
CNP-ase
MnCl2 100 nM
MnCl2 100 nM
MnCl2 100 nM
E14神経幹細胞に MnCl2 を処置することにより、GFAP 陽性アストロサイトへの分化誘導促進傾向が認められた。

Fig. 53

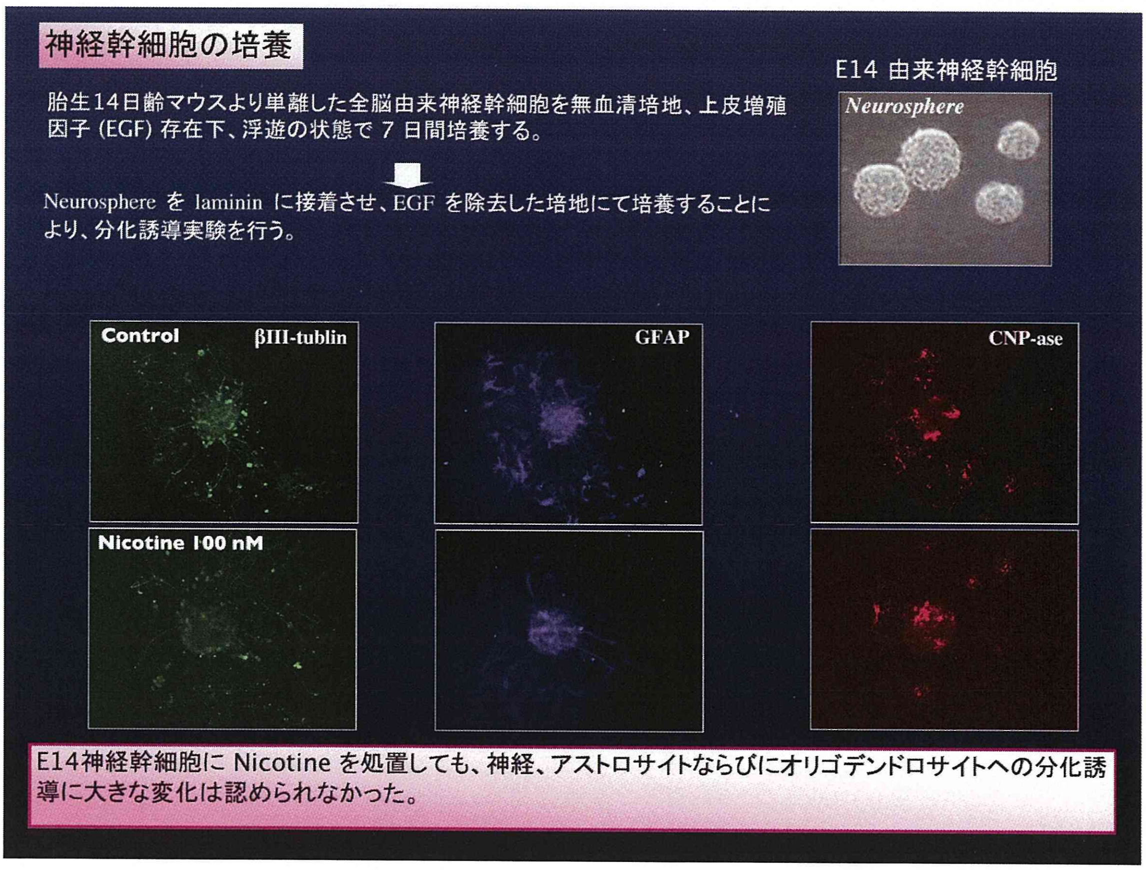

神経幹細胞の培養
E14 由来神経幹細胞
胎生14日齢マウスより単離した全脳由来神経幹細胞を無血清培地、上皮増殖因子 (EGF) 存在下、浮遊の状態で 7 日間培養する。
Neurosphere
Neurosphere を laminin に接着させ、EGF を除去した培地にて培養することにより、分化誘導実験を行う。
Control
βIII-tublin
GFAP
CNP-ase
Nicotine 100 nM
E14神経幹細胞に Nicotine を処置しても、神経、アストロサイトならびにオリゴデンドロサイトへの分化誘導に大きな変化は認められなかった。

Fig. 54

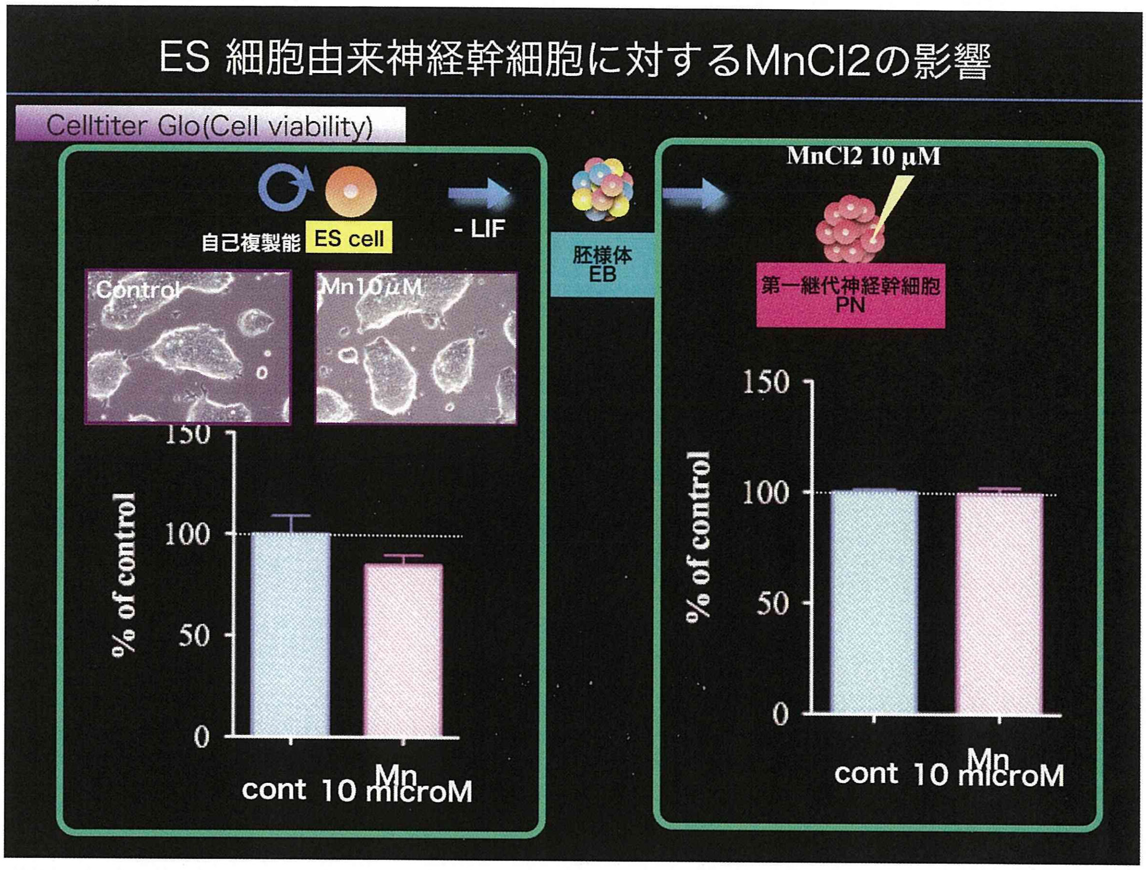

ES 細胞由来神経幹細胞に対するMnCl2の影響
Celltiter Glo(Cell viability)
自己複製能
ES cell
- LIF
胚様体
EB
MnCl2 10 μM
第一継代神経幹細胞
PN
Control
Mn10μM
% of control
150
100
50
0
cont
Mn
10 microM

Fig. 55

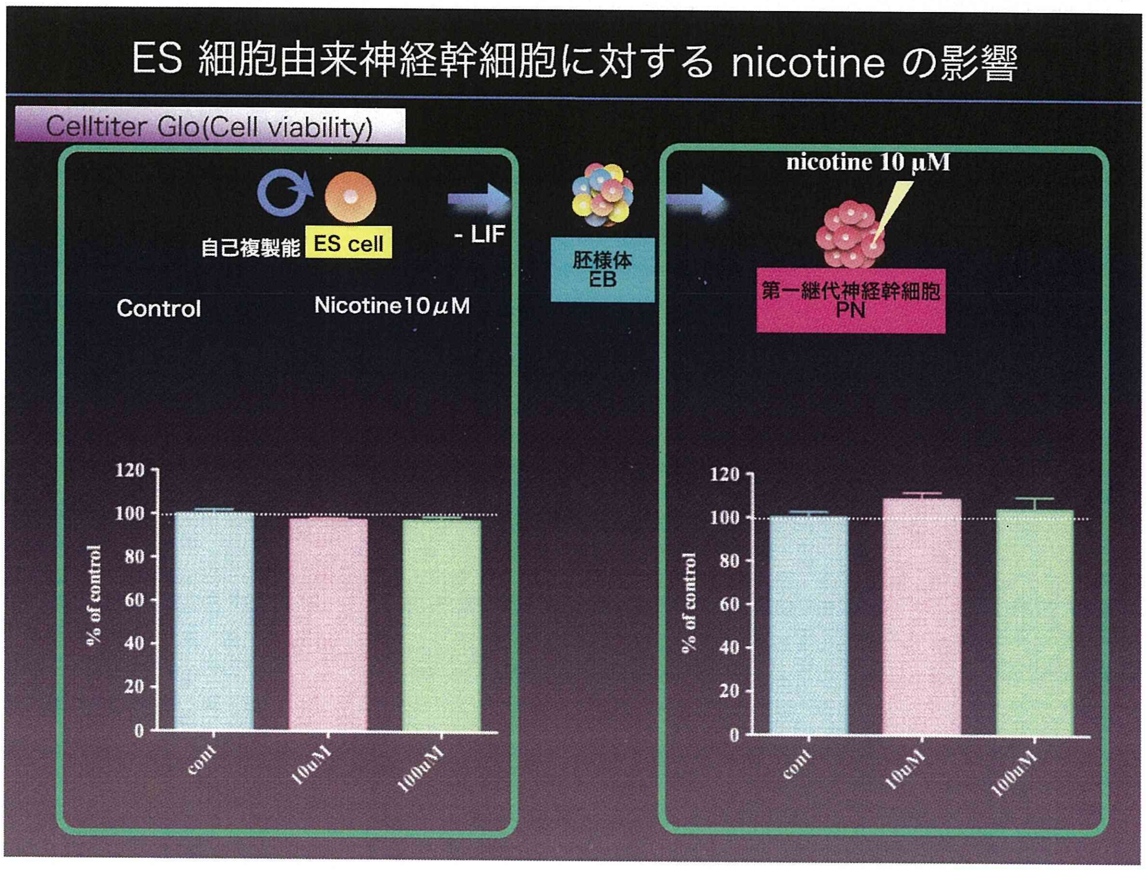
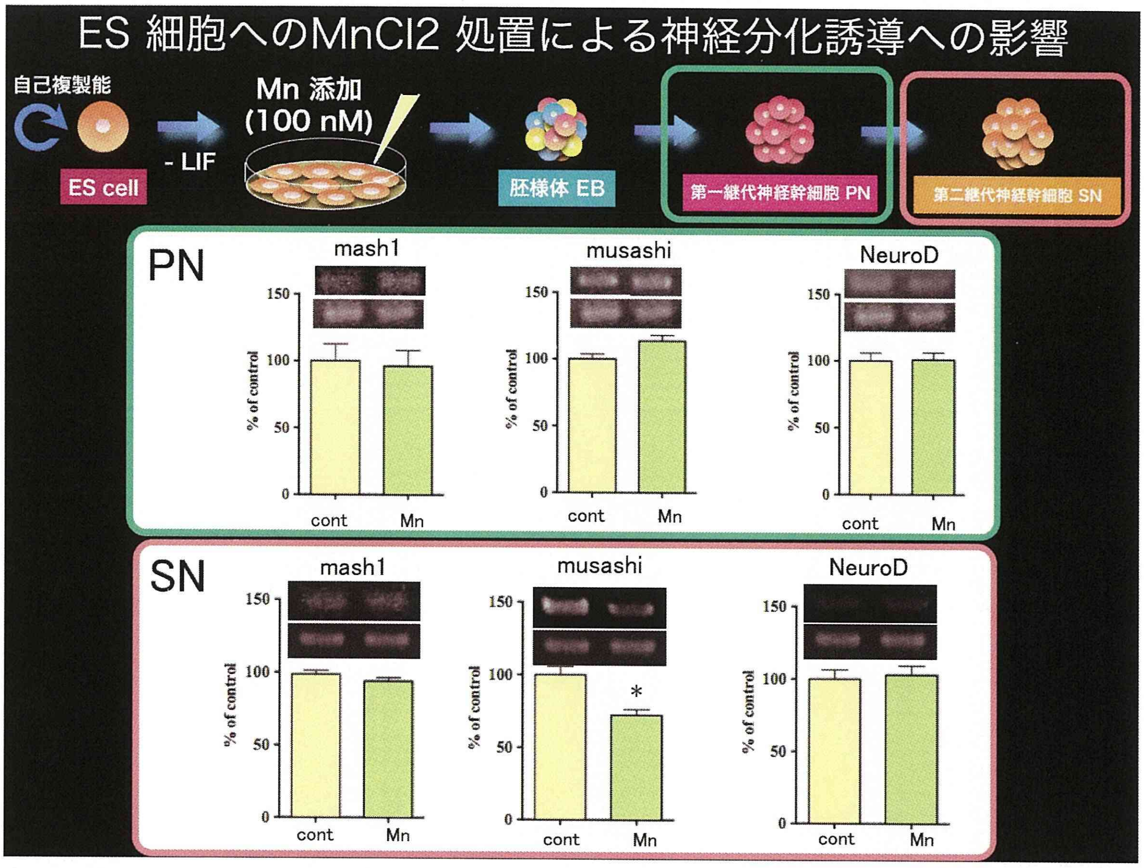
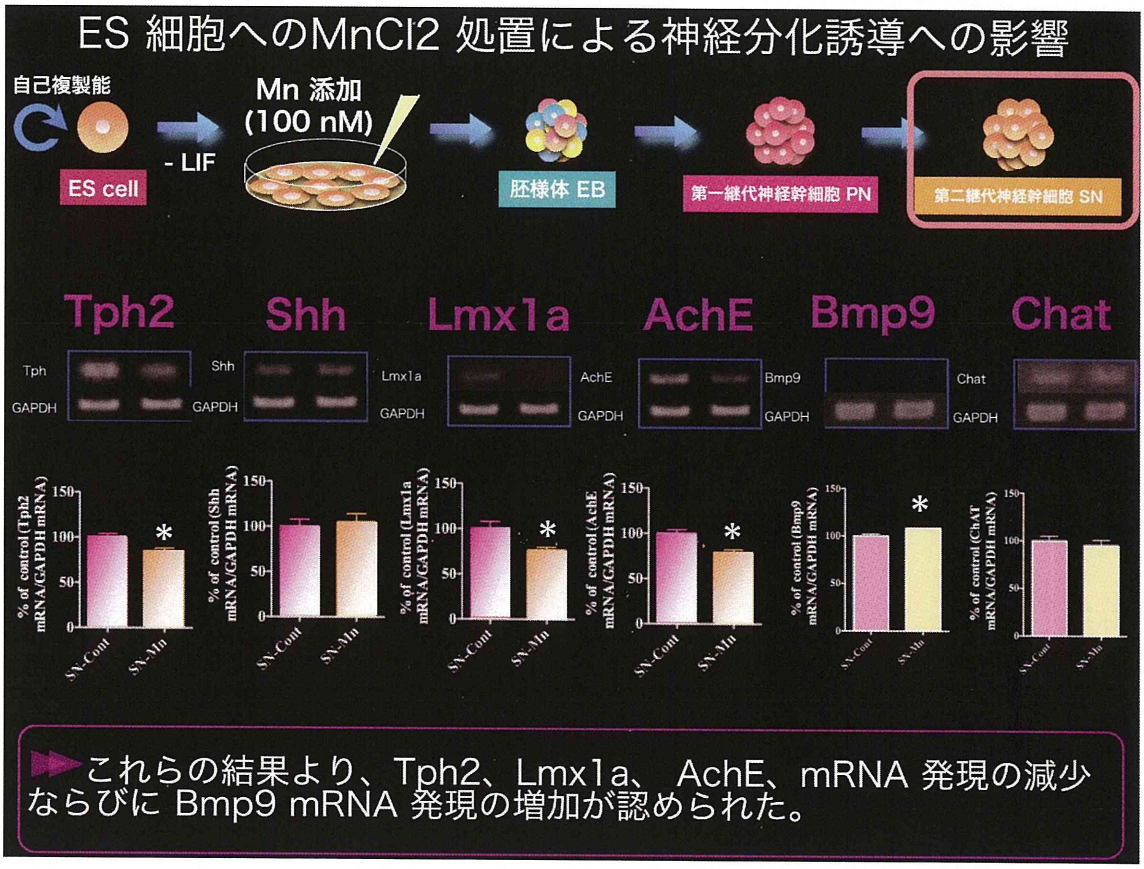

ES 細胞由来神経幹細胞に対する nicotine の影響
Celltiter Glo(Cell viability)
自己複製能
ES cell
- LIF
胚様体
EB
nicotine 10 μM
第一継代神経幹細胞
PN
Control
Nicotine10μM
% of control
120
100
80
60
40
20
0
cont
10uM
100uM

Fig. 56



ES 細胞へのMnCl2 処置による神経分化誘導への影響
自己複製能
ES cell
- LIF
Mn 添加
(100 nM)
胚様体 EB
第一継代神経幹細胞 PN
第二継代神経幹細胞 SN
PN
mash1
musashi
NeuroD
% of control
cont
Mn
SN
mash1
musashi
NeuroD
% of control
cont
Mn

Fig. 57



ES 細胞へのMnCl2 処置による神経分化誘導への影響
自己複製能
ES cell
- LIF
Mn 添加
(100 nM)
胚様体 EB
第一継代神経幹細胞 PN
第二継代神経幹細胞 SN
Tph2
Shh
Lmx1a
AchE
Bmp9
Chat
Tph
Shh
Lmx1a
AchE
Bmp9
Chat
GAPDH
SN-Cont
SN-Mn
これらの結果より、Tph2、Lmx1a、 AchE、mRNA 発現の減少
ならびに Bmp9 mRNA 発現の増加が認められた。

Fig. 58

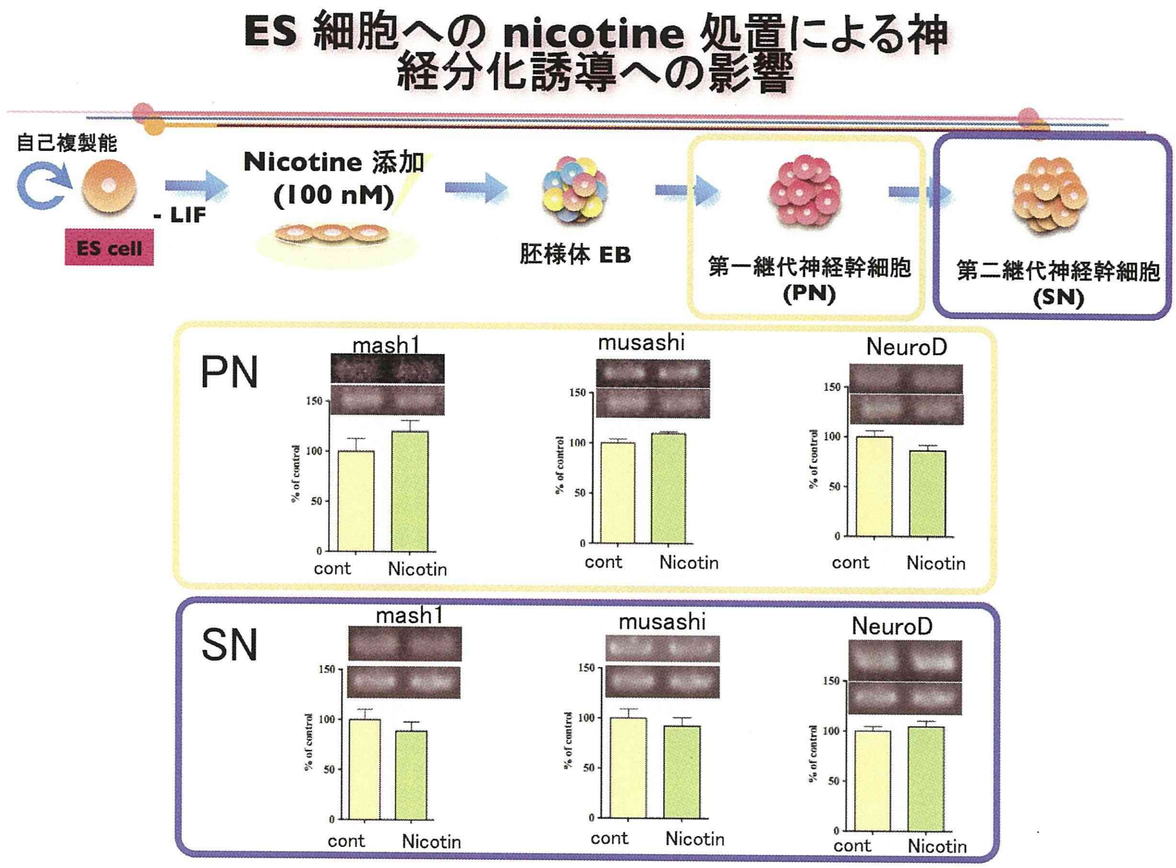
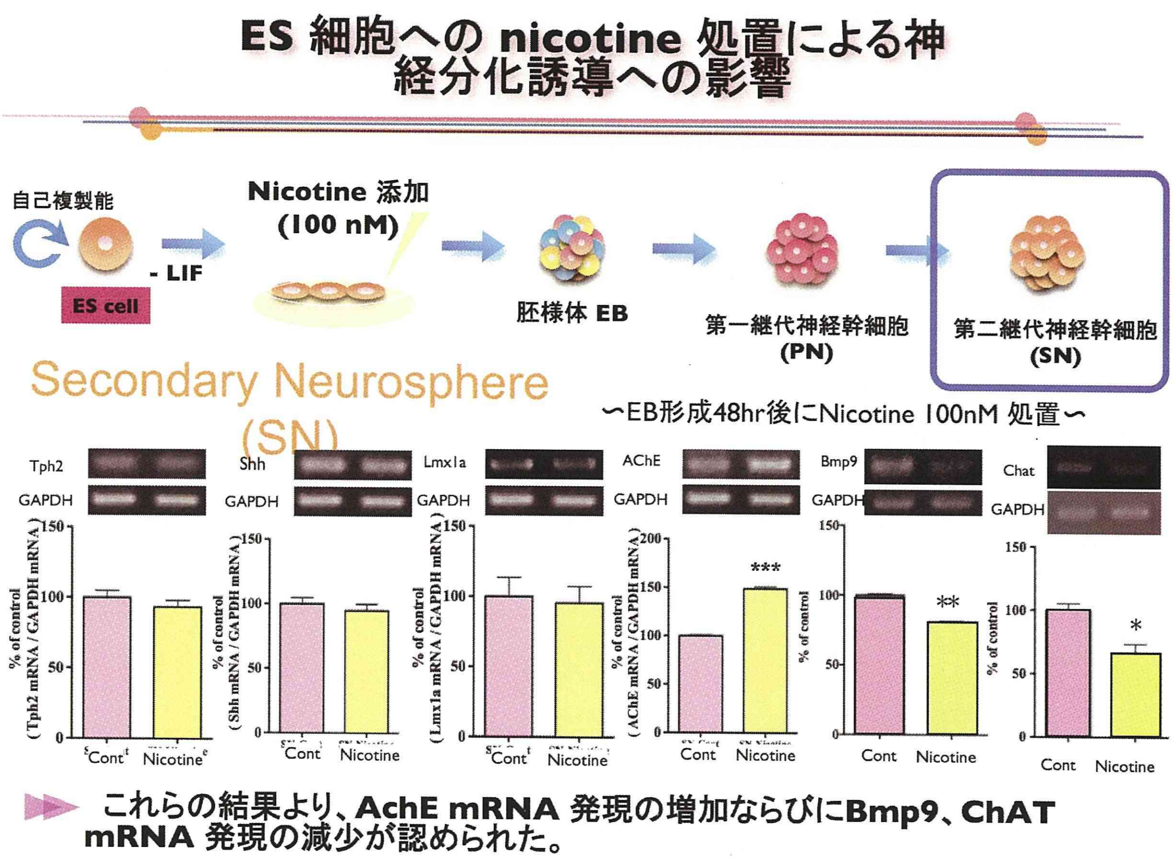

ES 細胞への nicotine 処置による神経分化誘導への影響
自己複製能
ES cell
- LIF
Nicotine 添加
(100 nM)
胚様体 EB
第一継代神経幹細胞
(PN)
第二継代神経幹細胞
(SN)
PN
mash1
musashi
NeuroD
% of control
cont
Nicotin
SN
mash1
musashi
NeuroD
% of control
cont
Nicotin

Fig. 59


ES 細胞への nicotine 処置による神経分化誘導への影響
自己複製能
ES cell
- LIF
Nicotine 添加
(100 nM)
胚様体 EB
第一継代神経幹細胞
(PN)
第二継代神経幹細胞
(SN)
Secondary Neurosphere
(SN)
〜EB形成48hr後にNicotine 100nM 処置〜
Tph2
GAPDH
Shh
GAPDH
Lmx1a
GAPDH
AChE
GAPDH
Bmp9
GAPDH
Chat
GAPDH
% of control (Tph2 mRNA / GAPDH mRNA)
% of control (Shh mRNA / GAPDH mRNA)
% of control (Lmx1a mRNA / GAPDH mRNA)
% of control (AChE mRNA / GAPDH mRNA)
% of control
Cont
Nicotine
***
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*
これらの結果より、AchE mRNA 発現の増加ならびにBmp9、ChAT mRNA 発現の減少が認められた。

Fig. 60

Table 53

Table 11. FACS analysis of effect of chlorpyrifos on the lymphocyte subpopulations

		Chlorpyrifos (ppm)			
		0	2.8	14	70
Female	n	4	4	4	4
PNW3					
[a]Spl: CD4$^+$/CD3$^+$		44.3 ± 1.0	45.8 ± 2.3	48.1 ± 2.9	49.9 ± 3.2*
Thy: CD4$^+$Foxp3$^+$		0.42 ± 0.02	0.42 ± 0.04	0.44 ± 0.30	0.85 ± 0.17*
PNW11					
Thy: CD4$^+$		9.5 ± 0.8	10.5 ± 0.7	12.3 ± 1.6**	12.6 ± 1.0**
Thy: CD4$^+$CD8a$^+$		86.4 ± 1.4	84.9 ± 1.7	83.4 ± 1.6	83.2 ± 1.2*
Male	n	4	4	4	4
PNW3					
Spl: CD4$^+$		4.5 ± 0.4	4.2 ± 0.7	4.0 ± 0.9	5.8 ± 0.2*
Thy: CD4$^+$IL17A$^+$		0.14 ± 0.05	0.10 ± 0.03	0.07 ± 0.02*	0.10 ± 0.03
PNW11					
Spl: CD4$^+$		16.3 ± 1.4	16.9 ± 1.8	17.8 ± 0.6	20.0 ± 1.3**
Spl: CD4$^+$CD25$^+$		1.9 ± 0.2	2.1 ± 0.2	2.4 ± 0.3*	2.4 ± 0.2*

Pregnant BALB/c mice (and their babies) were exposed to chlorpyrifos (0, 2.8, 14, and 70ppm) via food from gestational day (GD) 10 to postnatal week (PNW) 3. Exposure was ceased with leaning. At PNW3 and PNW11, mice were sacrificed to determine effects of the compound on the relative population of the lymphocyte subsets in the spleen (Spl) and thymus (Thy). Percent lymphocytes are shown ([a]Percent CD3 positive cells). Values are mean ± SD. Only statistically significant changes are shown. *$p<0.05$, **$p<0.01$ (Dunnett's test).

Table 54

CpG hyper- or hypo-methylated genes common in the thymus and spleen.

Hyper-methylated

GeneSymbol	Description	Ratio (Thy)	Ratio (Spl)	Chr. No	Region_info
Imp4	IMP4, U3 small nucleolar ribonucleoprotein, homolog (yeast)	77.18	15.84	1	INSIDE
Ppp1r7	protein phosphatase 1, regulatory (inhibitor) subunit 7	44.55	13.99	1	INSIDE
–	–	63.79	39.26	2	Unknown
Pax6	paired box gene 6	59.18	18.41	2	PROMOTER
Gtf2b	general transcription factor IIB	43.42	14.56	3	INSIDE
	protein tyrosine phosphatase 4a2	50.14	12.07	4	Unknown
Gng7	guanine nucleotide binding protein (G protein), gamma 7 subunit	72.42	33.84	10	PROMOTER
Utp18	UTP18, small subunit (SSU) processome component, homolog (yeast)	44.06	31.34	11	INSIDE
Ppm1d	protein phosphatase 1D magnesium-dependent, delta isoform	43.36	39.85	11	PROMOTER
Wdr35	WD repeat domain 35	57.08	32.56	12	PROMOTER
Wdr35	WD repeat domain 35	20.13	48.71	12	PROMOTER
Ext1	exostoses (multiple) 1	14.42	55.56	15	INSIDE
Slc25a23-Crb3	solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 23	33.49	48.29	17	DIVERGENT_PROMOTER
Tmem109	transmembrane protein 109	42.70	31.40	19	PROMOTER
Dpf2	D4, zinc and double PHD fingers family 2	41.57	20.16	19	INSIDE

Hypo-methylated

GeneSymbol	Description	Ratio (Thy)	Ratio (Spl)	Chr. No	Region_info
Speg	SPEG complex locus	0.09786	0.04088	1	INSIDE
LOC545667	–	0.03769	0.04435	4	INSIDE
6330407J23Rik	RIKEN cDNA 6330407J23 gene	0.04495	0.04115	10	INSIDE
Lrp1	low density lipoprotein receptor-related protein 1	0.06082	0.06016	10	INSIDE
Cdc42bpb	Cdc42 binding protein kinase beta	0.06177	0.05131	12	INSIDE
Pglyrp2	peptidoglycan recognition protein 2	0.01000	0.08583	17	INSIDE
Srrm2	serine/arginine repetitive matrix 2	0.01196	0.04570	17	INSIDE

Genes whose CpG methylation levels were hyper- or hypo-methylated more than 10 times both in the thymus and spleen after MMP exposure were selected. As up-regulated genes, only genes whose Normalized Ratio of CpG methylation exceeded 40 times in either tissue were listed. Chr. No, chromosome number. Region_info, information of the probing region of the interested genes.

Table 55

Effect of MMP on the CpG methylation of immune-related genes in thymus and spleen.

Thymus

GeneSymbol	Description	Normalized ratio	Chr. No	Region_info
<Hyper-methylated>				
Edg3	endothelial differentiation, sphingolipid G-protein-coupled receptor, 3	17.59	13	INSIDE
Hlxb9	homeobox gene HB9	12.25	5	INSIDE
Tap2	transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	7.088	17	PROMOTER
<Hypo-methylated>				
Vav1	vav 1 oncogene	0.01545	17	PROMOTER
Sh2b2	SH2B adaptor protein 2	0.08780	5	INSIDE
Pglyrp2	peptidoglycan recognition protein 2	0.1028	17	INSIDE
Smad3	MAD homolog 3 (Drosophila)	0.1857	9	PROMOTER

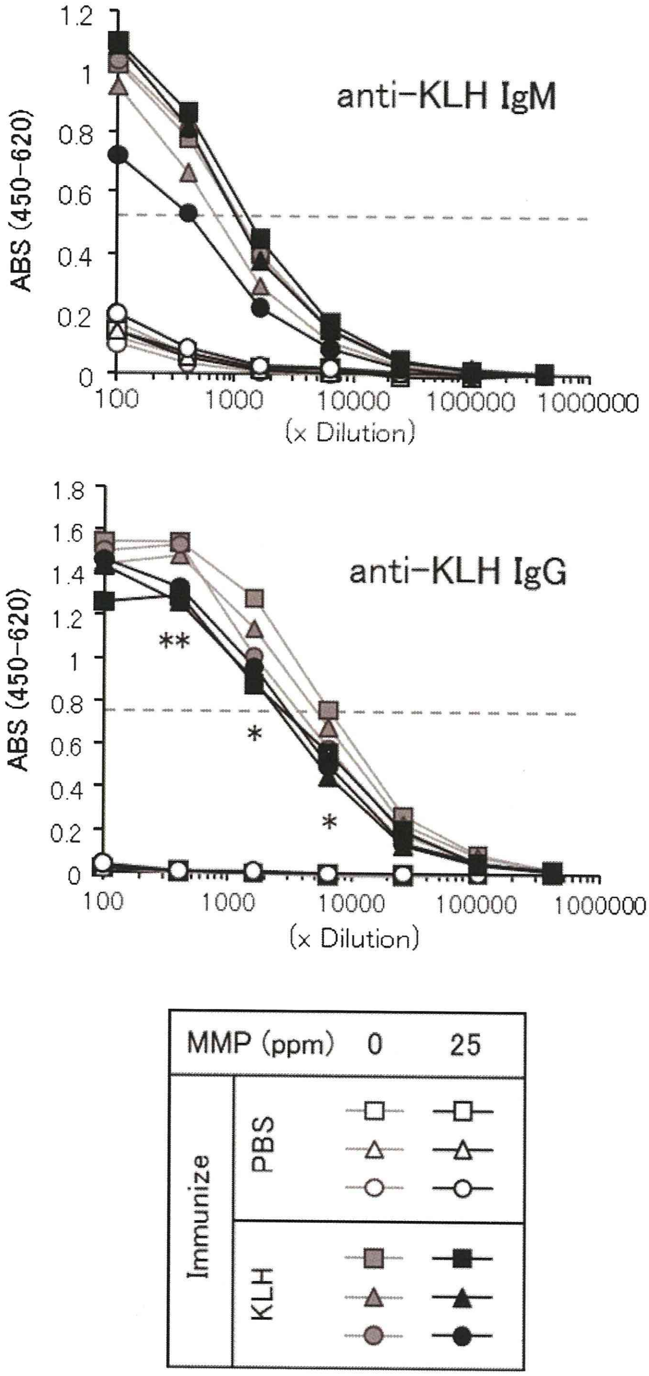
Spleen

GeneSymbol	Description	Normalized ratio	Chr. No	Region_info
<Hyper-methylated>				
Psmb9-Tap1	proteosome (prosome, macropain) subunit, beta type 9 (large multifunctional peptidase 2)	44.61	17	DIVERGENT_PROMOTER
Fcgr1	Fc receptor, IgG, high affinity I	34.20	3	DOWNSTREAM
Bcl3	B-cell leukemia/lymphoma 3	30.68	7	INSIDE
C1qb	complement component 1, q subcomponent, beta polypeptide	27.94	4	INSIDE
Cblb	Casitas B-lineage lymphoma b	20.17	16	INSIDE
Prok2	prokineticin 2	20.09	6	PROMOTER
Tgfb1	transforming growth factor, beta 1	18.91	7	INSIDE
Bcl10	B-cell leukemia/lymphoma 10	14.93	3	PROMOTER
Tmem142a	transmembrane protein 142A	14.66	5	INSIDE
Arl6ip2	ADP-ribosylation factor-like 6 interacting protein 2	12.51	17	INSIDE
Smad1	MAD homolog 1 (Drosophila)	10.71	8	PROMOTER
Vav1	vav 1 oncogene	8.085	17	PROMOTER
Hlxb9	homeobox gene HB9	6.765	5	PROMOTER
<Hypo-methylated>				
Cd14	CD14 antigen	0.01771	18	INSIDE
Ncf1	neutrophil cytosolic factor 1	0.03753	5	INSIDE
Pglyrp2	peptidoglycan recognition protein 2	0.06301	17	INSIDE

Among the genes whose CpG methylation level was hyper- or hypo-methylated more than four times after exposure to 25ppm MMP, entries which have immune-related* biological process in the Gene ontology were selected. If multiple probe IDs were hit for an identical gene, only one probe ID was selected by following priorities: 1) an entry whose Region_info is 'PROMOTER', 2) an entry whose fold change of the Normalized_ratio is the largest.

*GO:50776(regulation of immune response); GO:16064(immunoglobulin mediated immune response); GO:45087(innate immune response); GO:6959(humoral immune response); GO:6955(immune response); GO:42088(T-helper 1 type immune response); GO:2455(humoral immune response mediated by circulating immunoglobulin); GO:6954(inflammatory response).

Fig. 61



Effect of MMP on the antibody generation to KLH.

As thymus-dependent antigen, 1 mg/ml of keyhole limpet hemocyanin (KLH) was intraperitoneally injected to the female offspring at postnatal day (PND) 22 and PND 36. PBS was injected as a vehicle control. Serum KLH-specific IgM and IgG were measured with ELISA. *$p<0.05$, **$p<0.01$ (Student's t-test, n=3).