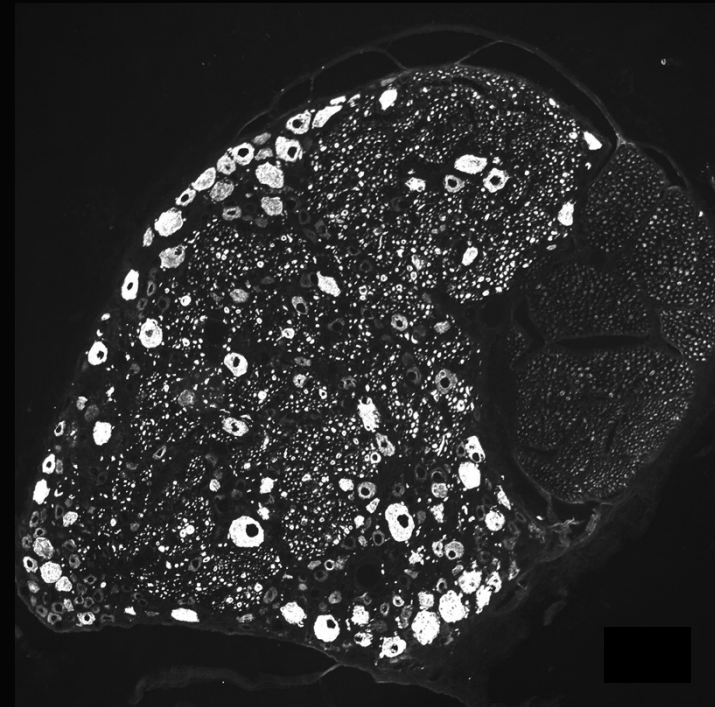
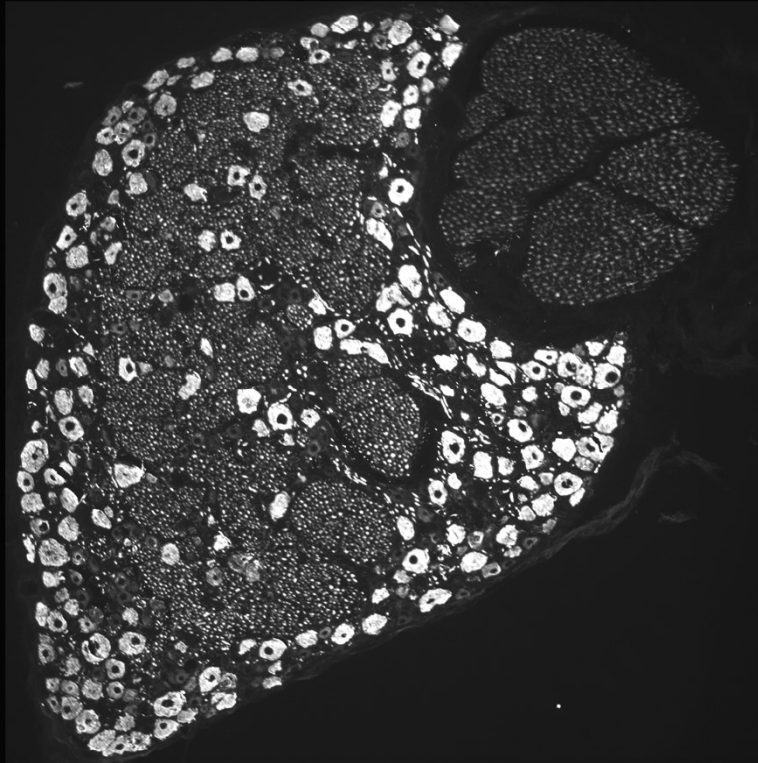


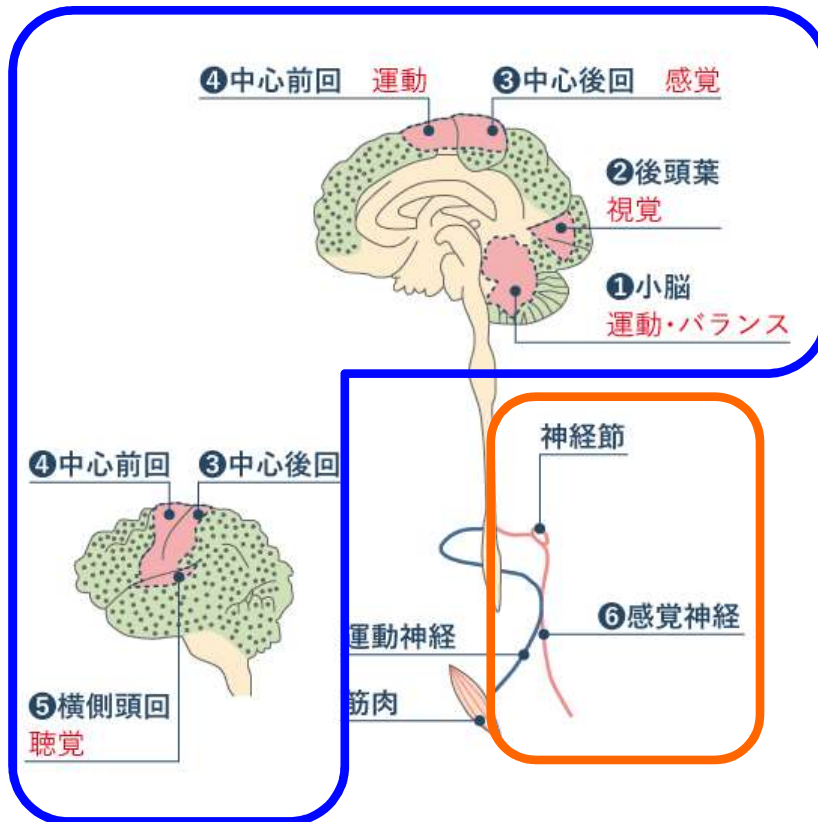
メチル水銀による末梢感覚神経障害と その回復機構



2024年8月23日
日化協LRI研究報告会

東京薬科大学薬学部
篠田 陽

メチル水銀による神経障害



運動失調 (大脳運動野・小脳・末梢感覚神経)

構音障害 (小脳・末梢感覚神経)

視野狭窄 (大脳視覚野)

感覚障害 (大脳感覚野・末梢感覚神経)

聴力障害 (大脳聴覚野)

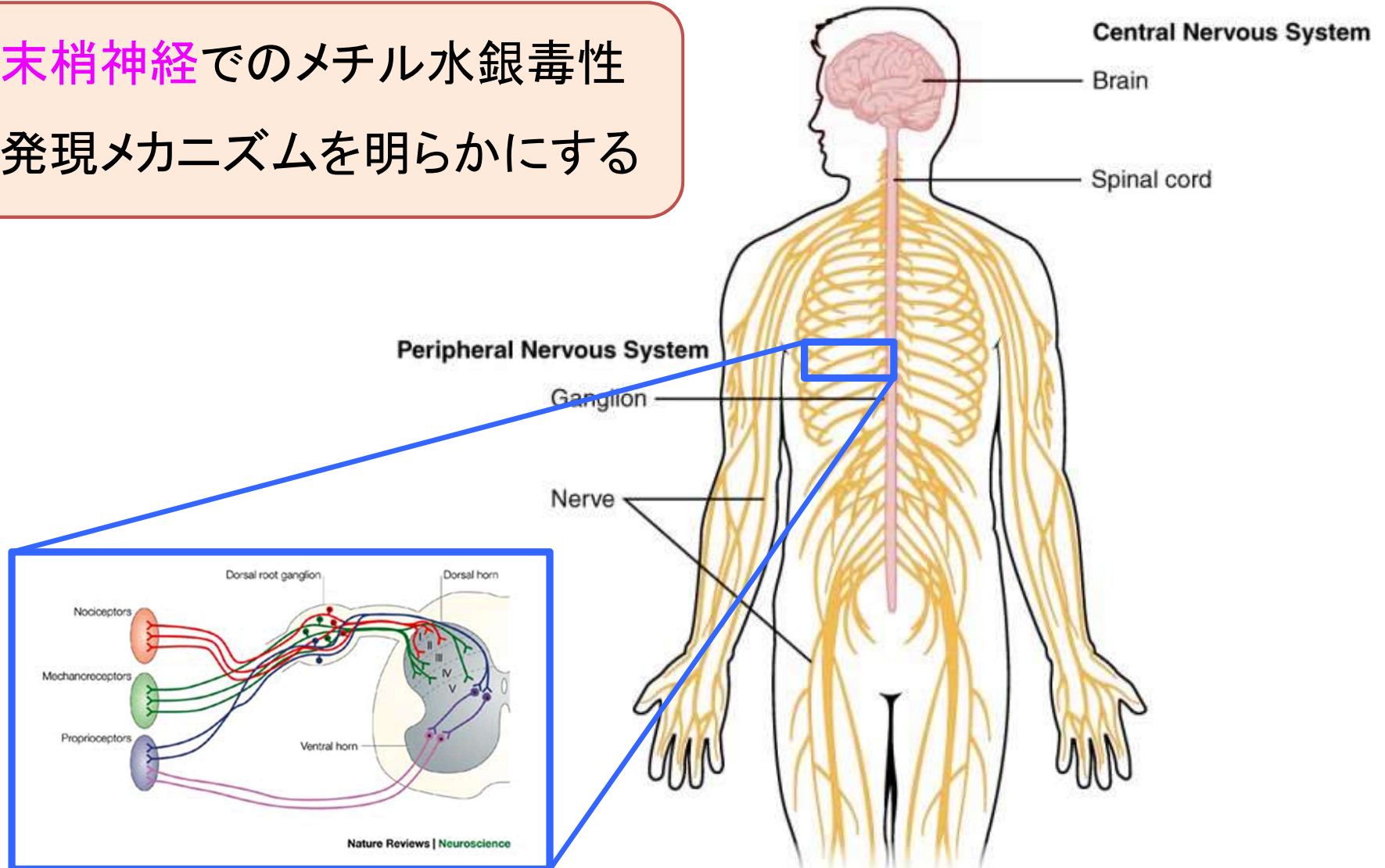
運動障害 (大脳運動野・小脳)

水俣病情報センターHP
Eto, *Toxicol. Pathol.* (1997)
一部改変

末梢神経系におけるメチル水銀毒性の
発現機構はほとんど研究されていない

末梢神経におけるメチル水銀毒性発現機構の解明

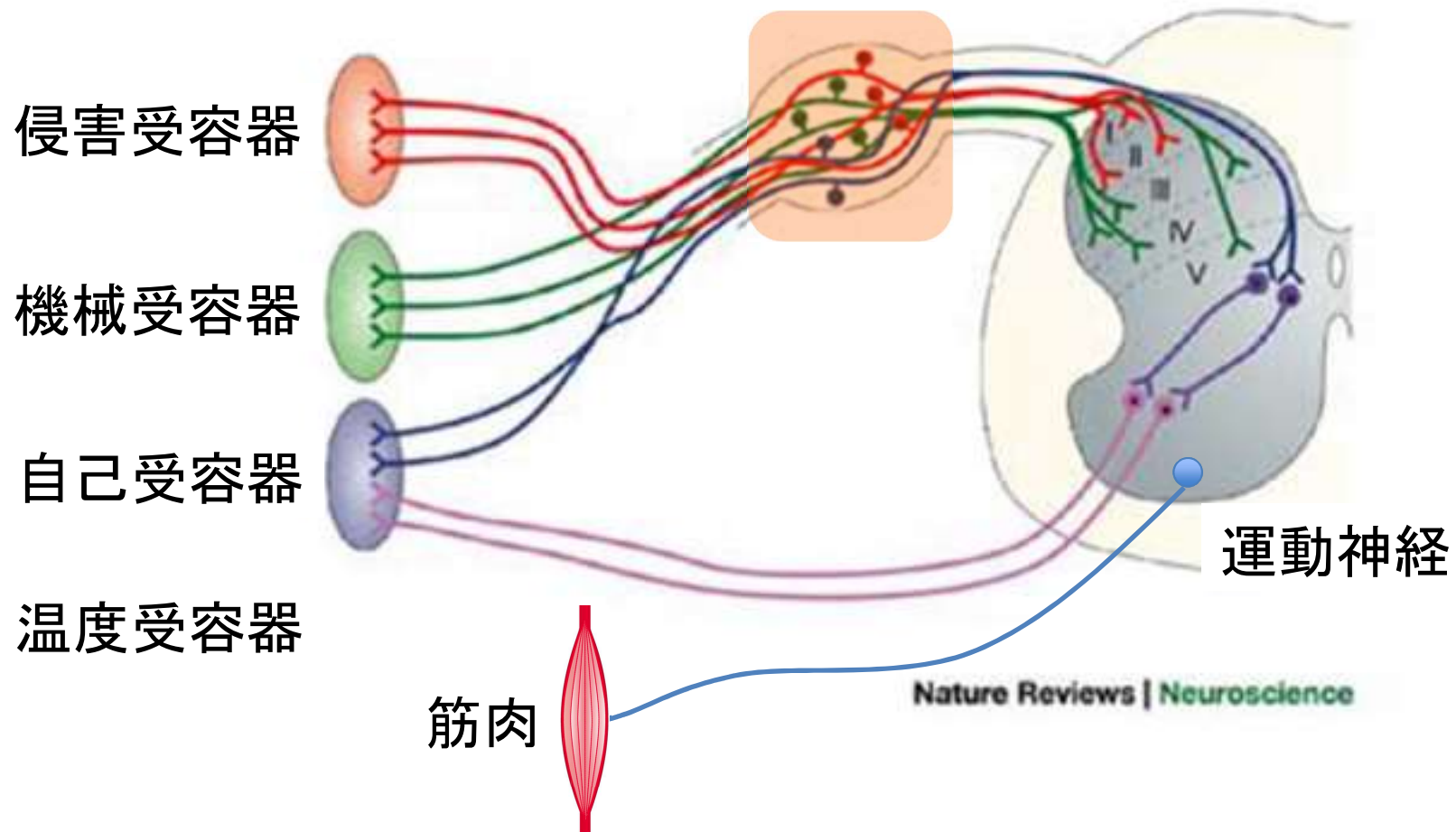
末梢神経でのメチル水銀毒性
発現メカニズムを明らかにする



末梢神経の構造

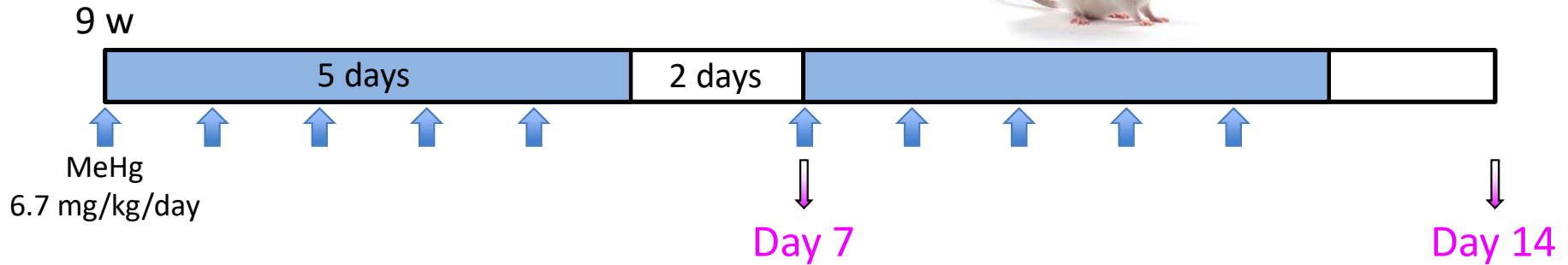
感覚神経

後根神経節 (Dorsal root ganglion: DRG)



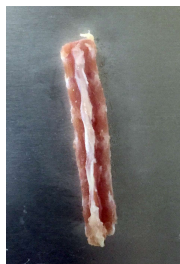
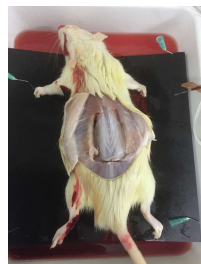
Histological analysis of MeHg-exposed rat DRG

Minamata disease model rat (male Wistar rat)

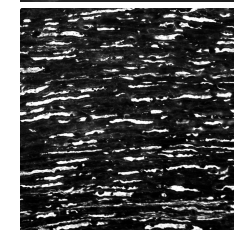
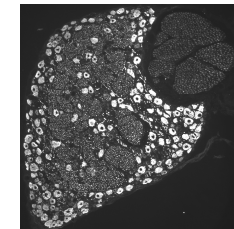
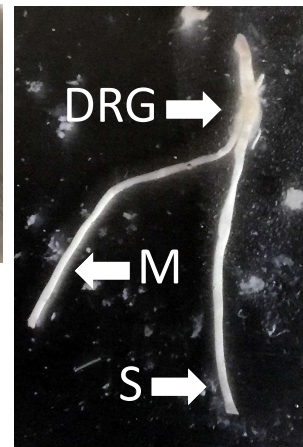


DRG preparation for IHC

4% PFA fixation → Preparation of DRG and nerve fibers → Immunostaining



Motor (M) and
Sensory (S) fiber



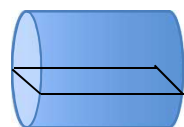
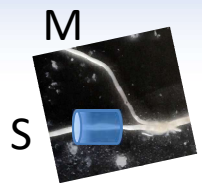
Shinoda *et al.*, 2019

Sensory nerve

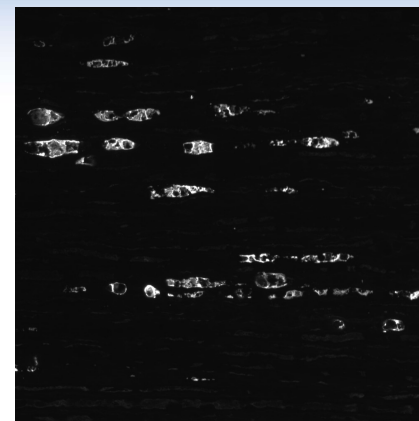
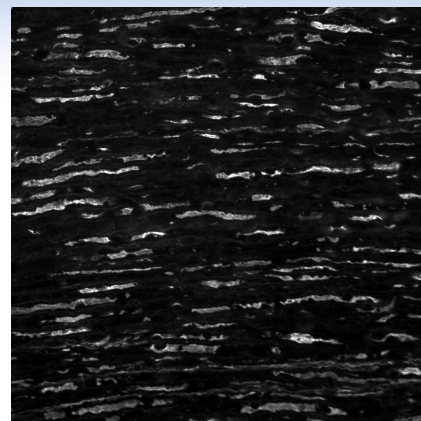
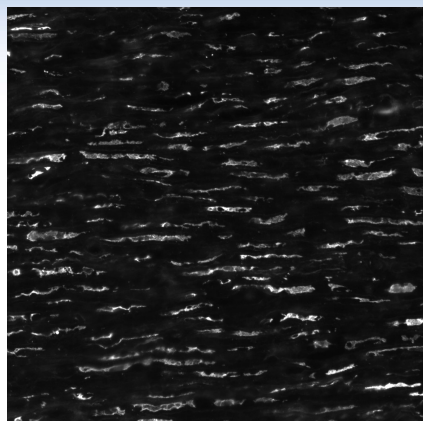
Cont

MeHg Day 7

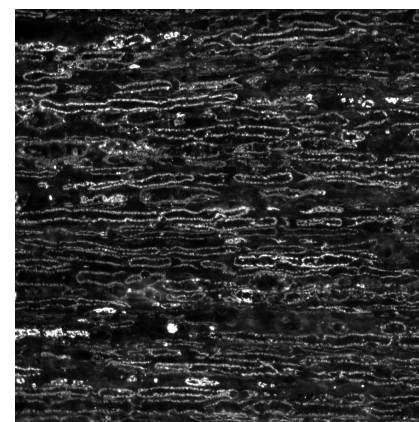
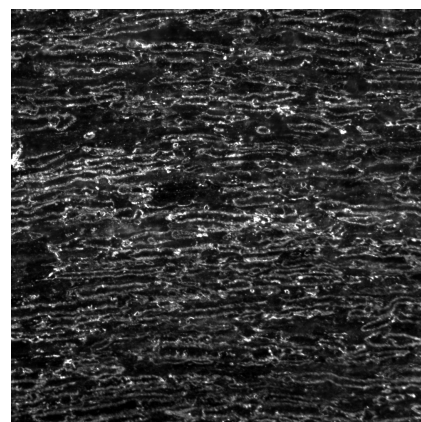
MeHg Day 14



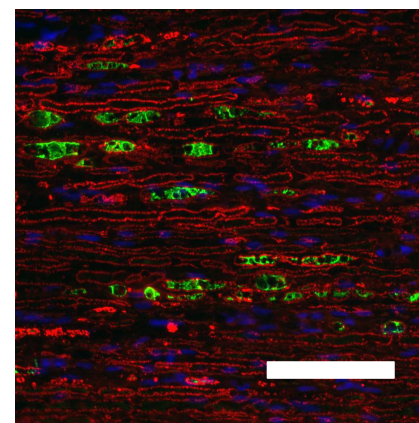
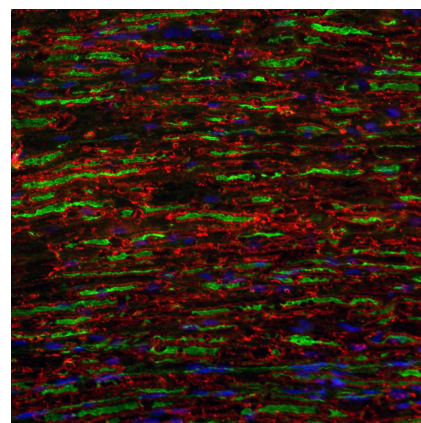
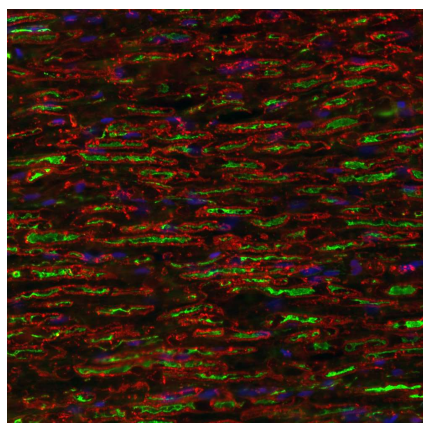
Neurofilament



MBP



Merge



50 μ m

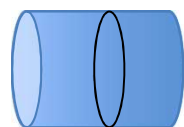
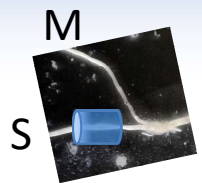
Shinoda *et al.*, 2019

Sensory nerve

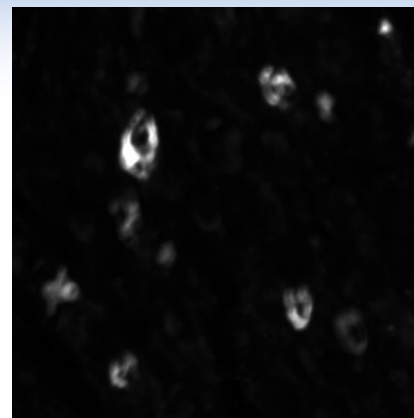
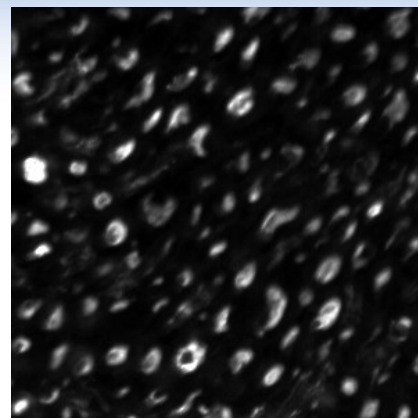
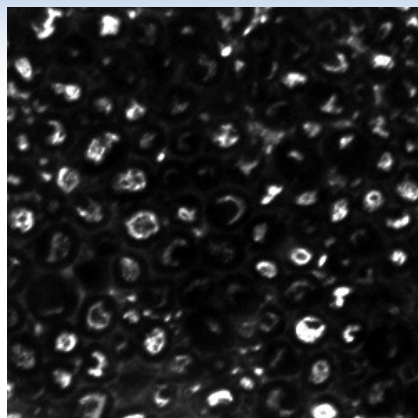
Cont

MeHg Day 7

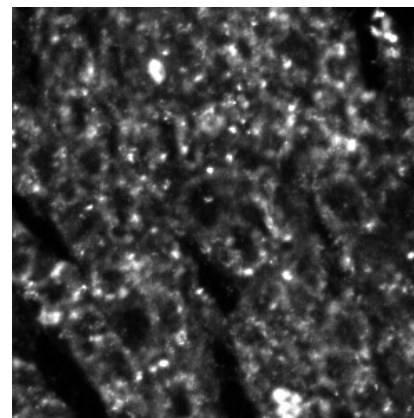
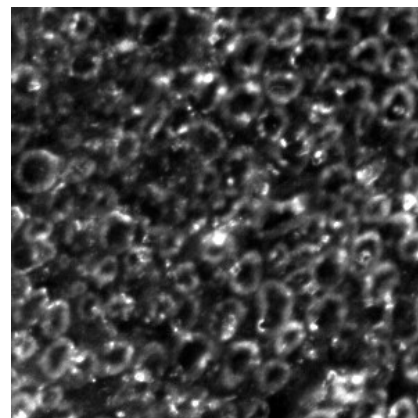
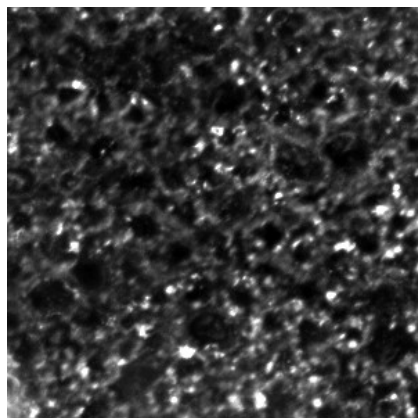
MeHg Day 14



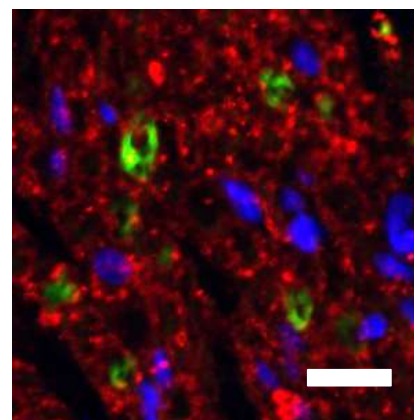
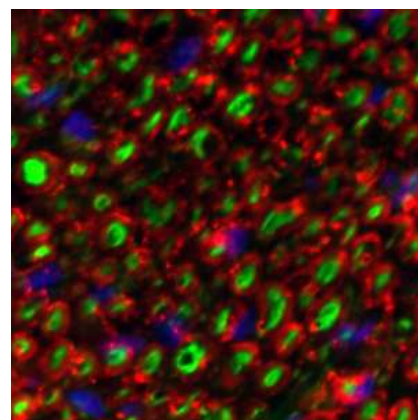
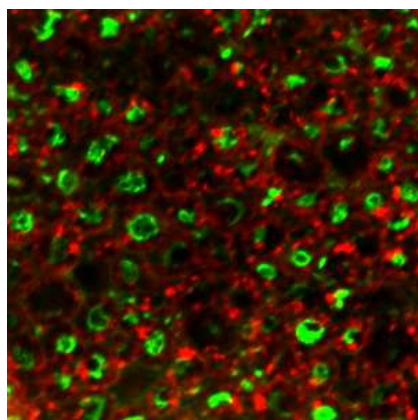
Neurofilament



MBP



Merge



10 μ m

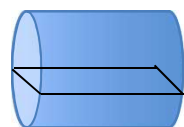
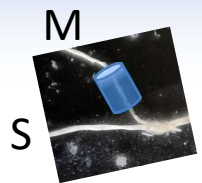
Shinoda *et al.*, 2019

Motor nerve

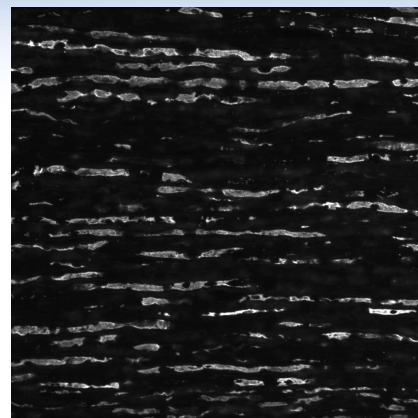
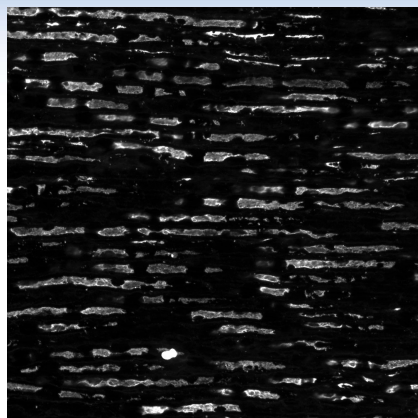
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MeHg Day 7

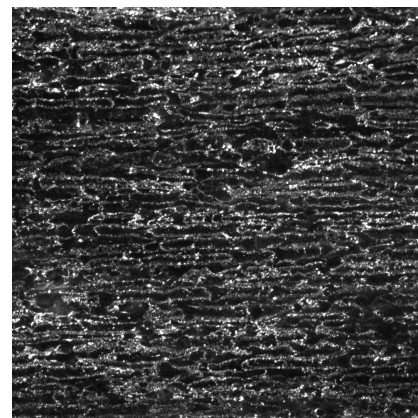
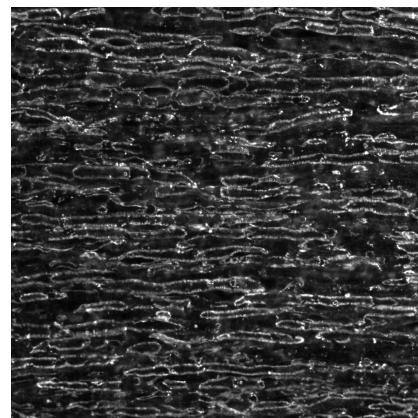
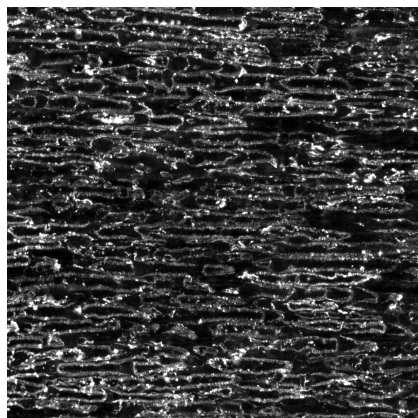
MeHg Day 14



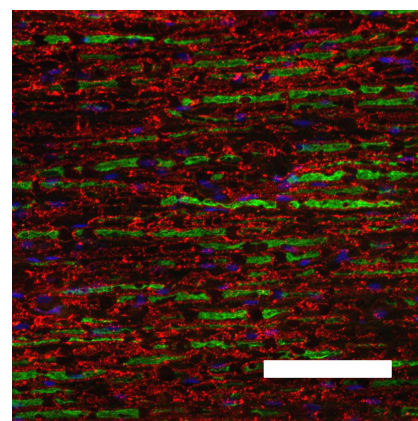
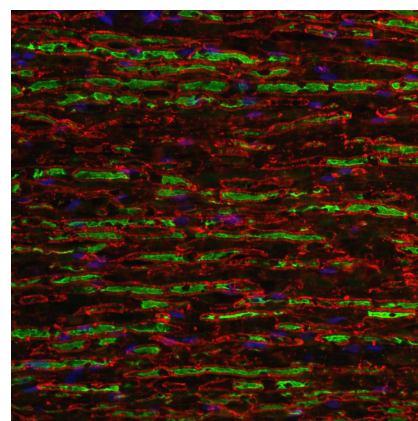
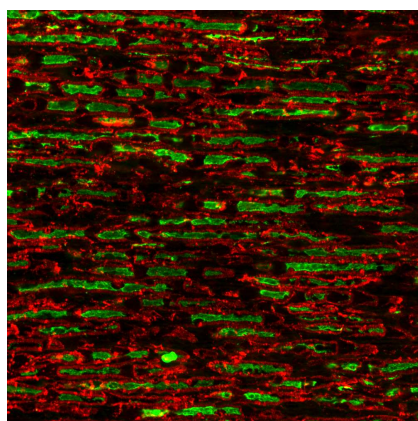
Neurofilament



MBP



Merge



50 μ m

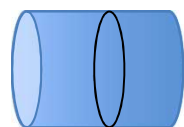
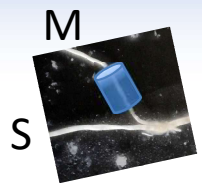
Shinoda *et al.*, 2019

Motor nerve

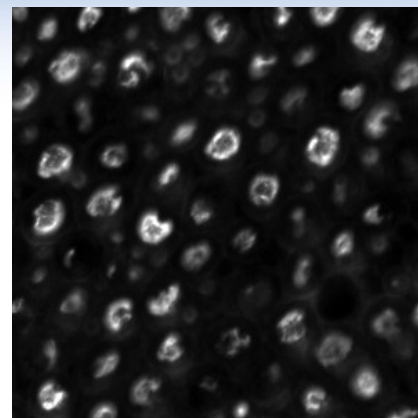
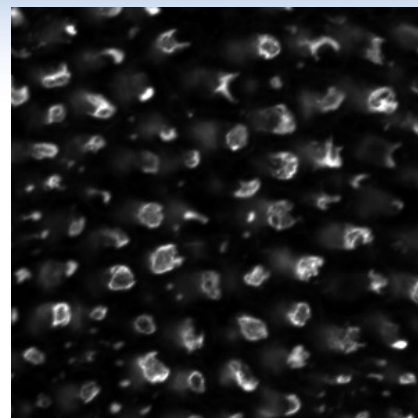
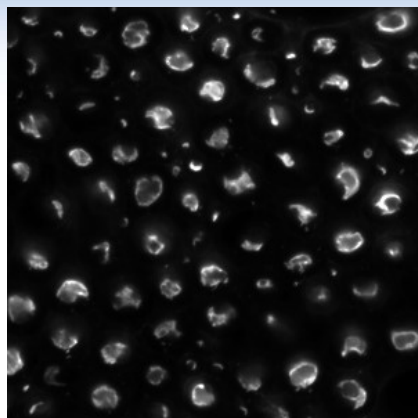
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MeHg Day 7

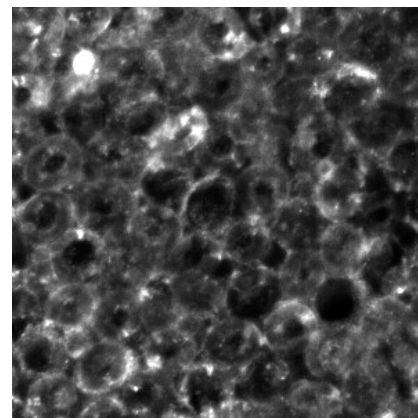
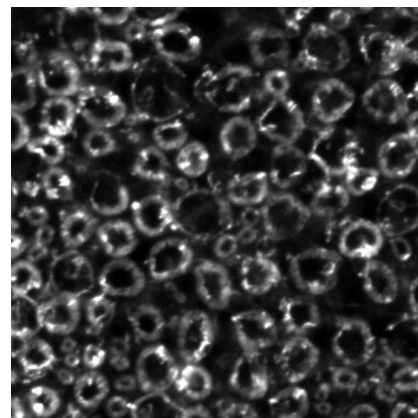
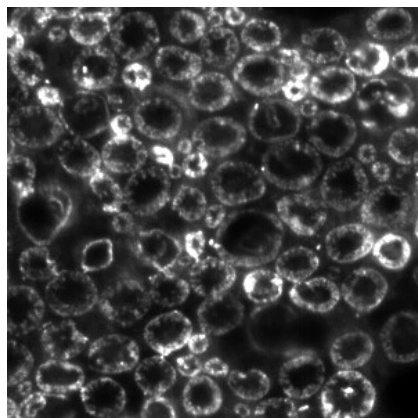
MeHg Day 14



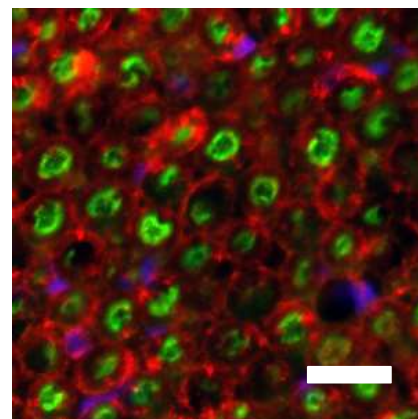
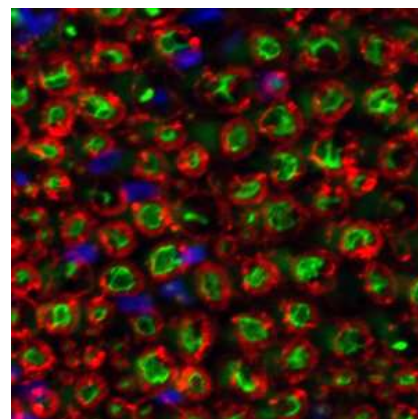
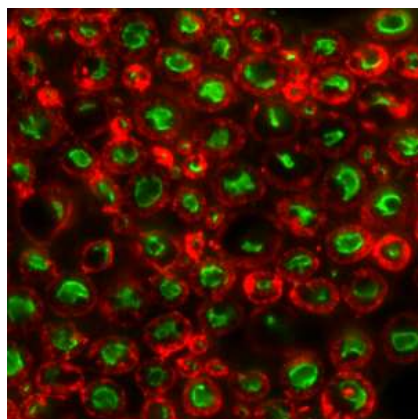
Neurofilament



MBP



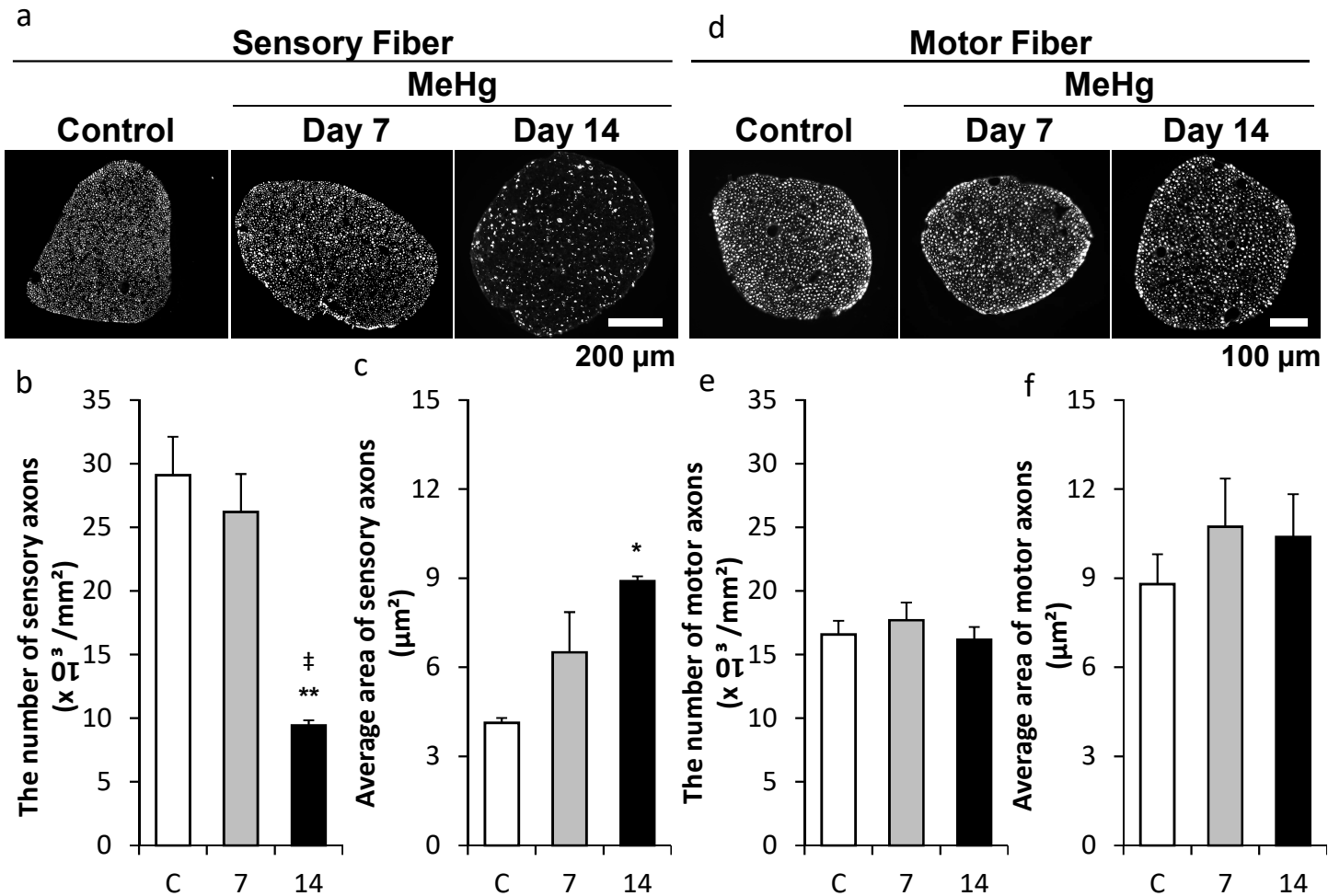
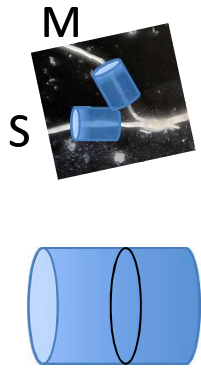
Merge



10 μ m

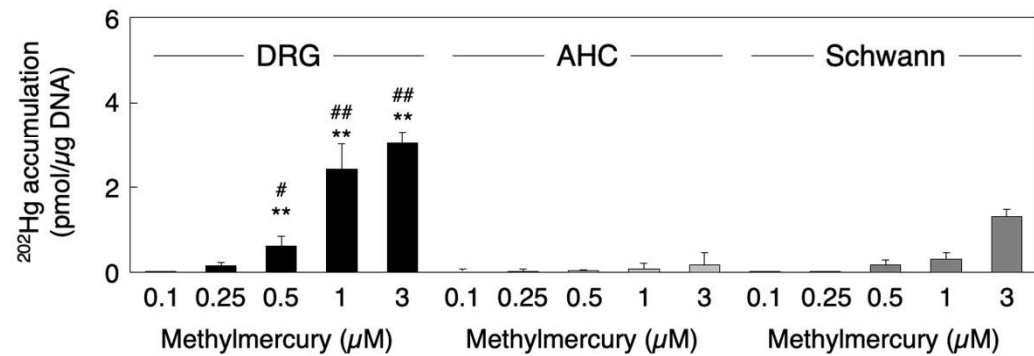
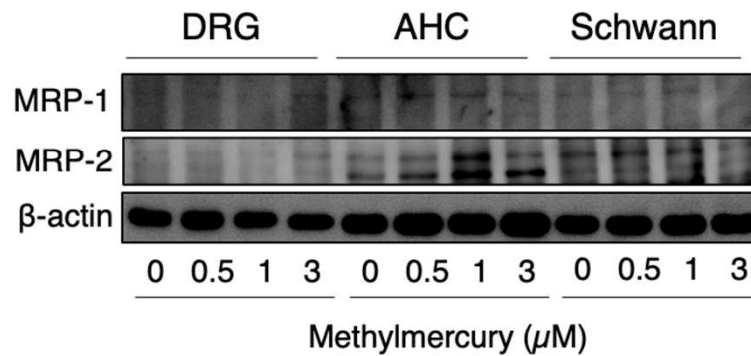
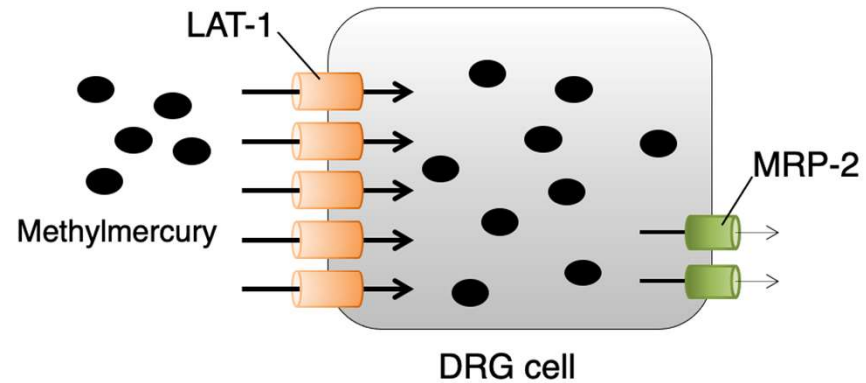
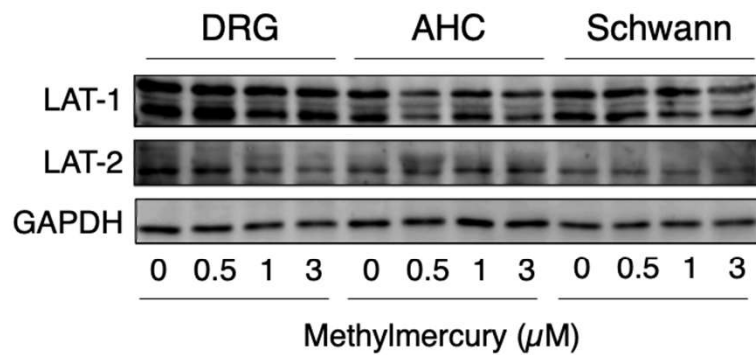
Shinoda *et al.*, 2019

Sensory but not motor nerve was injured by MeHg



n = 3, **p < 0.01 *p < 0.05 vs control, †p < 0.01 vs Day 7, one-way ANOVA post-hoc Tukey-Kramer

メチル水銀による感覚神経選択的な傷害



** $p < 0.01$ vs. AHC, # $p < 0.05$ and ## $p < 0.01$ vs. Schwann cells.

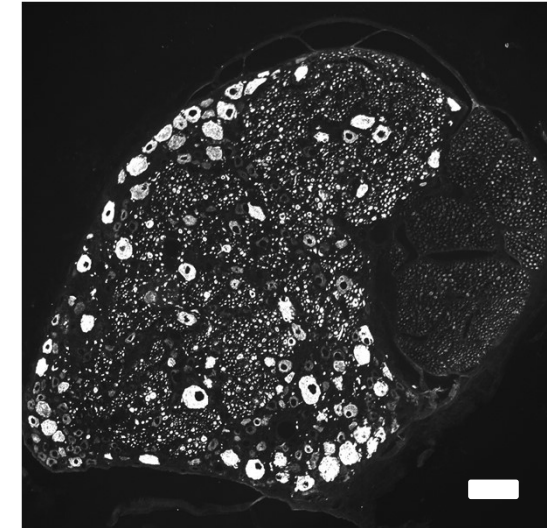
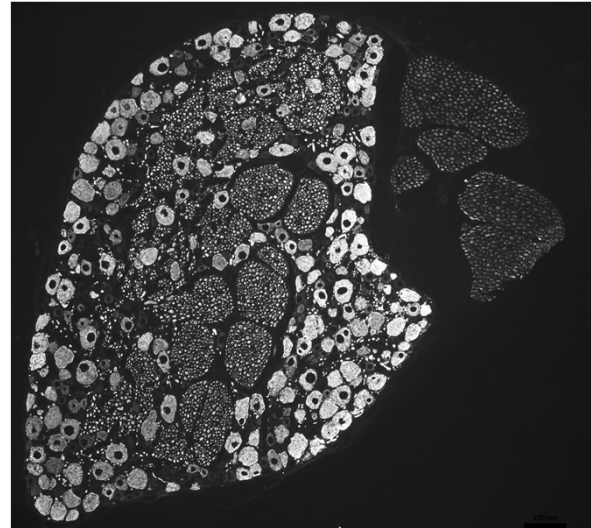
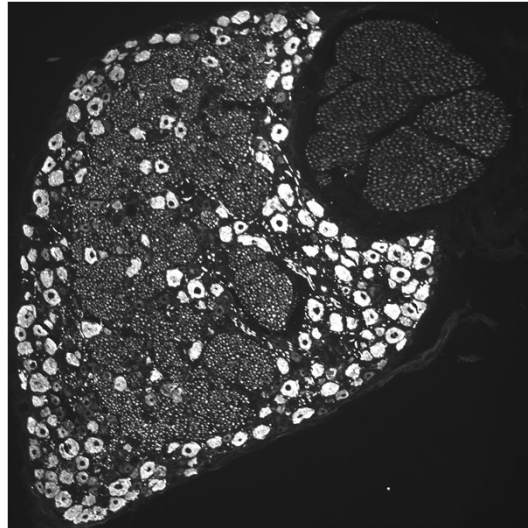
Middle size neurons in DRG were specifically degenerated by MeHg

MeHg

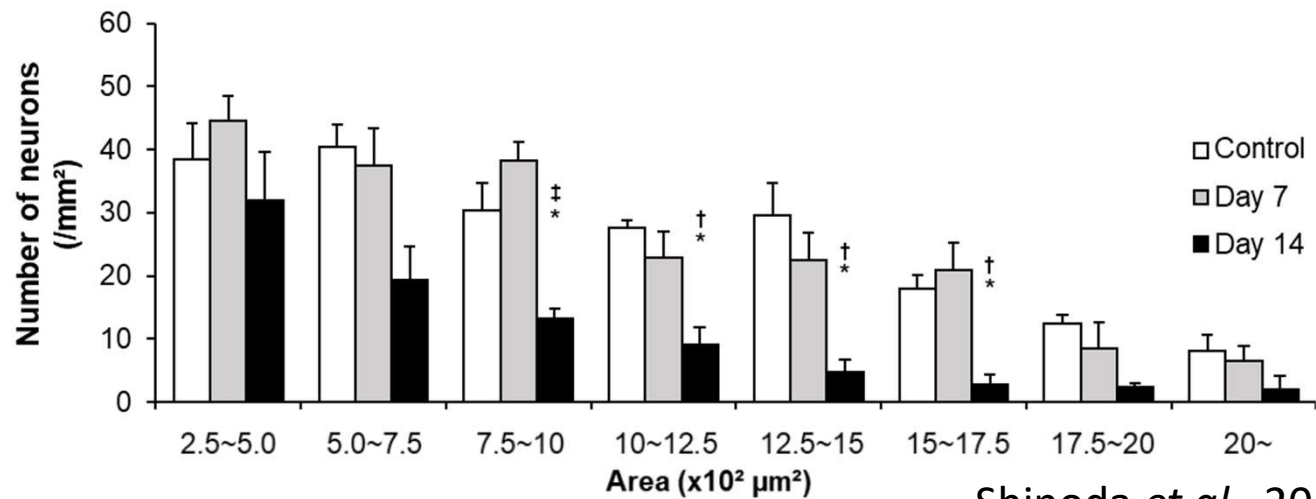
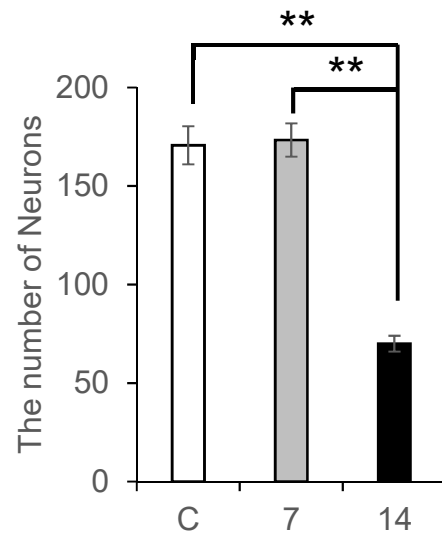
Control

Day 7

Day 14



100 μm



Shinoda *et al.*, 2019

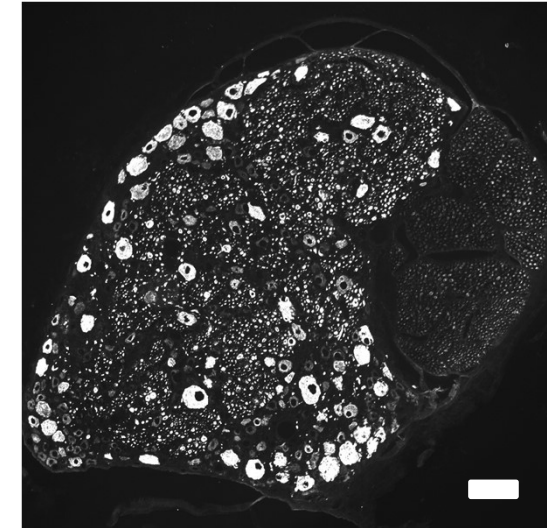
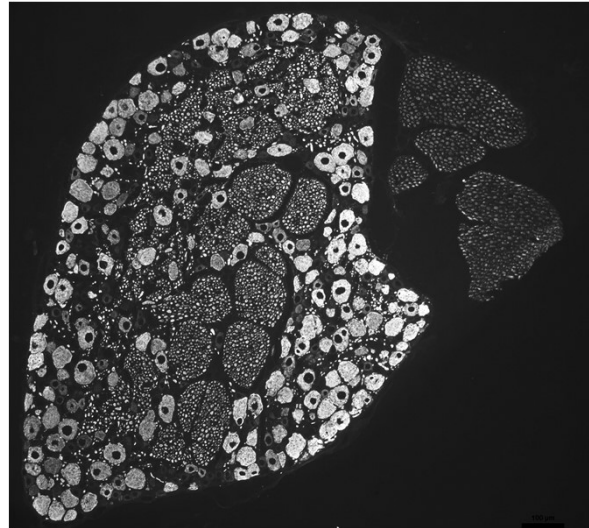
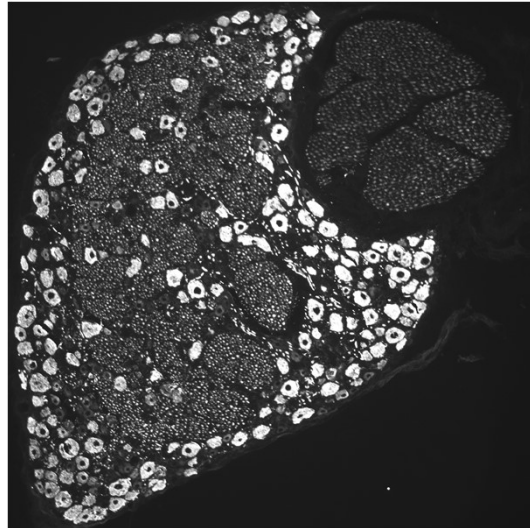
Middle size neurons in DRG were specifically degenerated by MeHg

MeHg

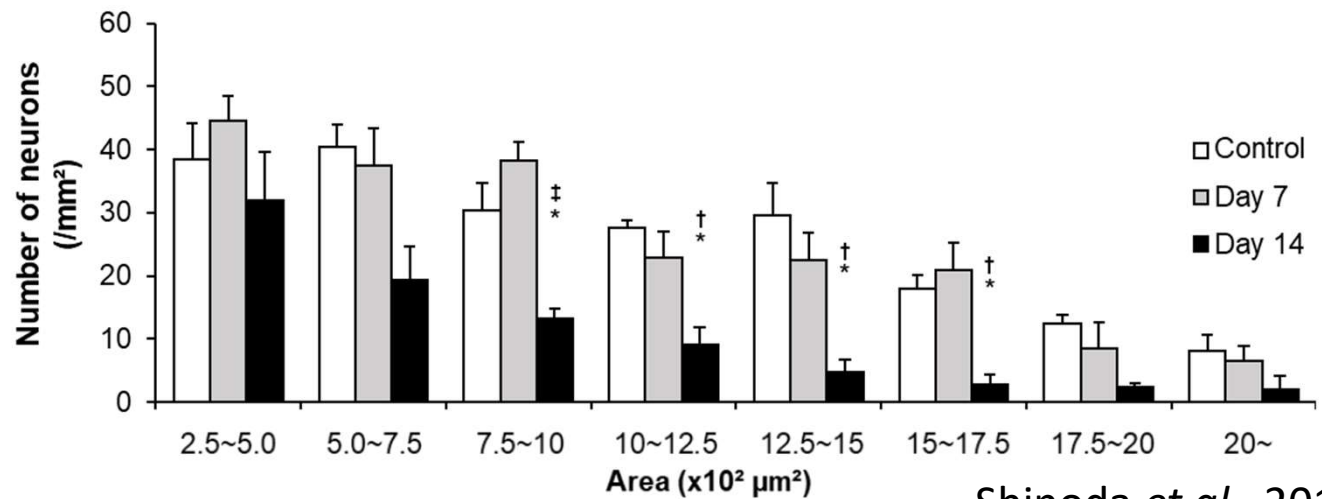
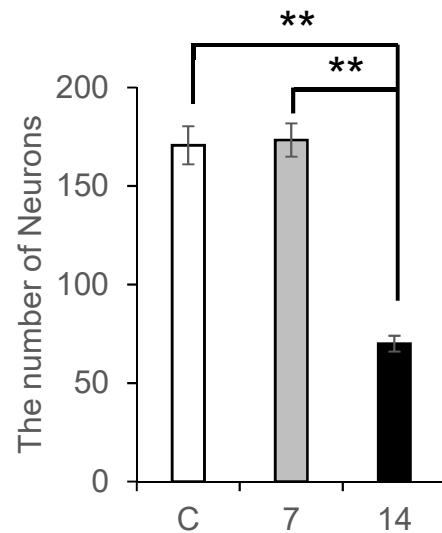
Control

Day 7

Day 14



100 μm

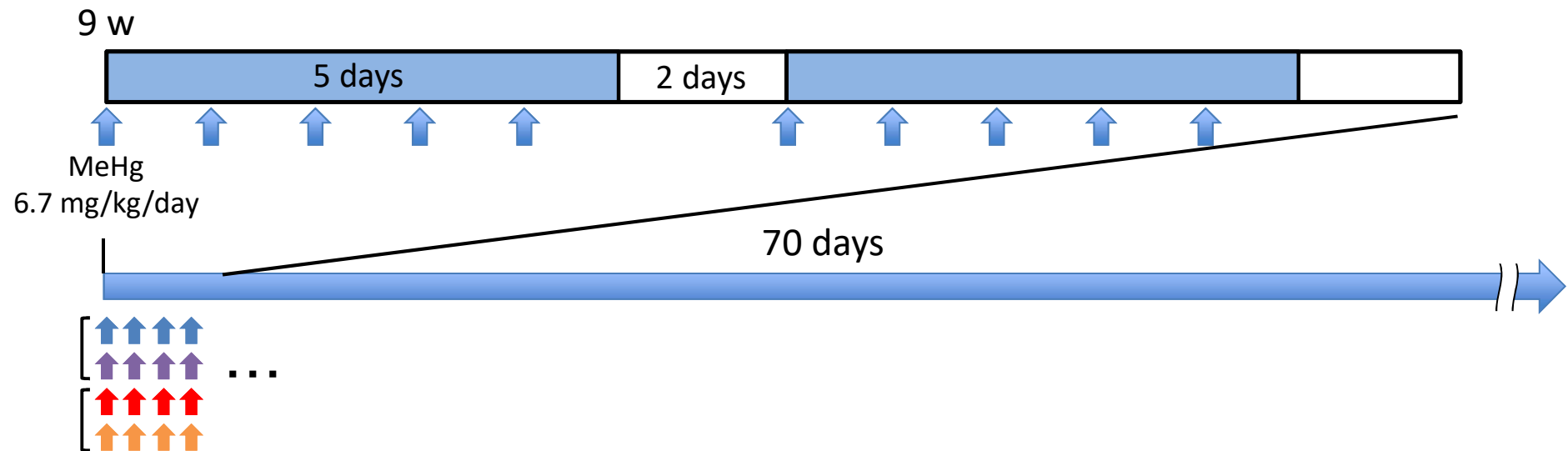


Shinoda *et al.*, 2019

動物個体を用いた刺激応答解析による メチル水銀毒性の各種感覚モダリティ特異性解析

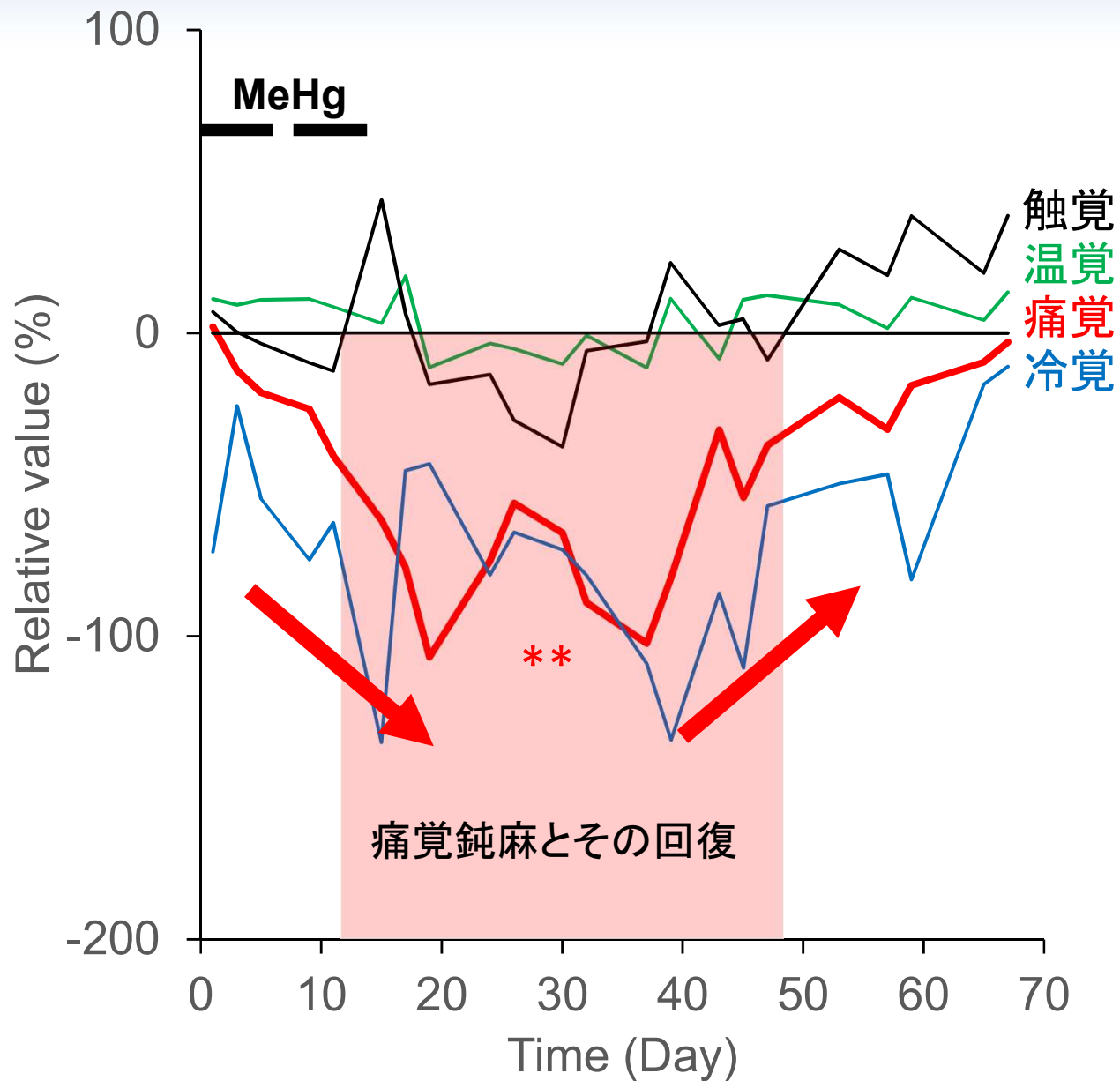


Minamata disease model rat (male Wistar rat)



- ↑ Tail dipping test (cold) (冷感刺激・冷覚)
- ↑ Foot shock test (侵害刺激・痛覚)
- ↑ Tail flick test (温感刺激・温覚)
- ↑ von Frey test (圧力刺激・触覚)

メチル水銀曝露の種々感覚モダリティへの影響

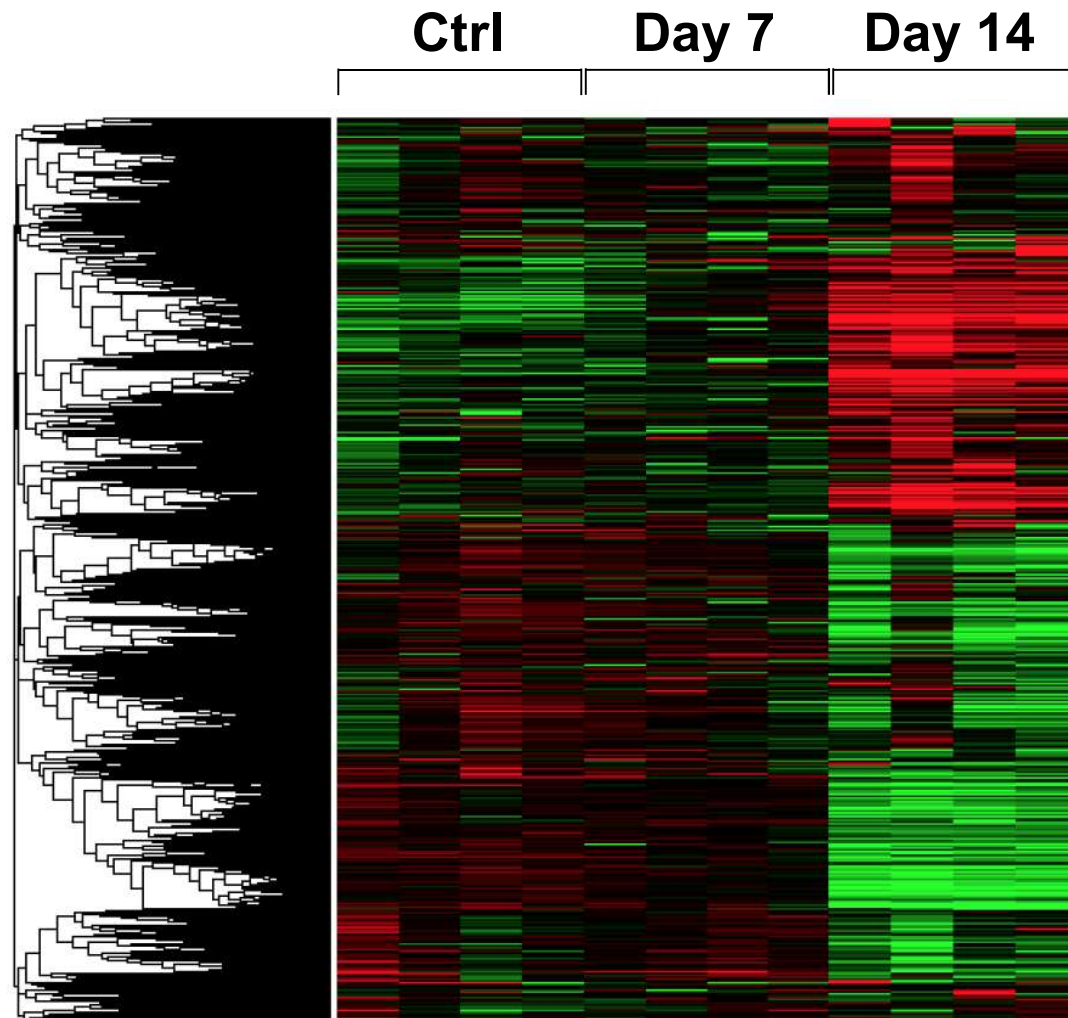
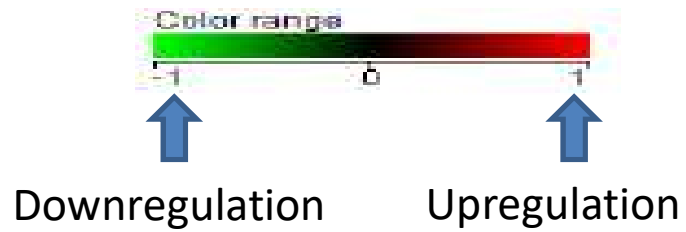


DRGにおける神経細胞は11種類知られている

NF1	NF2	NF3	NF4	NF5	NP1	NP2	NP3	PEP1	PEP2	TH
LDHB CACNA1H TRKB ^{high} NECAB2	LDHB CACNA1H TRKB ^{low} CALB1 RET	LDHB TRKC ^{high} FAM19A1 RET	LDHB TRKC ^{low} PV SPP1 CNTNAP2	LDHB TRKC ^{low} PV SPP1 CNTNAP2	PLXNC1 ^{high} P2X3 GFRA2 MRGPRD	PLXNC1 ^{high} P2X3 TRKA CGRP MRGPRA3	PLXNC1 ^{high} P2X3 <u>SST</u>	TRKA CGRP KIT TAC1 PLXNC1 ^{low}	<u>TRKA</u> CGRP KIT CNTNAP2 <u>FAM19A1</u>	PIEZO2 ^{high} VGLUT3 GFRA2
LTMRs		Proprioceptors			Nonpeptidergic			Peptidergic		C-LTMRs
Unmyelinated		Myelinated			Unmyelinated			Myel.		Unmyel.
<u>NEFH</u>	NEFH RET	NEFH RET	NEFH ASIC1 RUNX3	NEFH ASIC1 RUNX3	RET TRPA1 TRPC3 NAV1.8/9	RET TRPV1 TRPA1 TRPC3 NAV1.8/9	RET TRPV1 TRPA1 TRPC3 NAV1.8/9	TRPV1 NAV1.8/9	NEFH NAV1.8/9	RET TRPA1 NAV1.8/9

Usoskin *et al.*, 2015

DNAマイクロアレイによるDRGの遺伝子変動パターン



メチル水銀投与により変動した遺伝子数

Total RNA from DRG
control, Day 7 and 14 (N = 4)
> 2 folds change
One-way ANOVA P < 0.05

Day 7

Up 957 genes

Down 444 genes

Total 1401 genes

Day 14

Up 5888 genes

Down 8970 genes

Total 14858 genes



The genes changed in Day 7 were analyzed
by DAVID pathway analysis

メチル水銀による免疫応答や炎症応答のシグナル惹起

44 chart records

[Download File](#)

Sublist	Category	Term	RT	Genes	Count	%	P-Value	Benjamini
<input type="checkbox"/>	KEGG_PATHWAY	Cytokine-cytokine receptor interaction	RT		35	3.3	1.5E-8	3.9E-6
<input type="checkbox"/>	KEGG_PATHWAY	Tuberculosis	RT		28	2.6	1.6E-6	5.9E-5
<input type="checkbox"/>	KEGG_PATHWAY	Phagosome	RT		27	2.5	2.0E-5	5.7E-4
<input type="checkbox"/>	KEGG_PATHWAY	HTLV-1 infection	RT		27	2.5	8.3E-3	8.7E-2
<input type="checkbox"/>	KEGG_PATHWAY	MAPK signaling pathway	RT		24	2.2	1.4E-2	1.2E-1
<input type="checkbox"/>	KEGG_PATHWAY	Osteoclast differentiation	RT		23	2.2	1.4E-6	6.1E-5
<input type="checkbox"/>	KEGG_PATHWAY	Chemokine signaling pathway	RT		23	2.2	2.2E-4	4.9E-3
<input type="checkbox"/>	KEGG_PATHWAY	Herpes simplex infection	RT		23	2.2	3.2E-3	3.9E-2
<input type="checkbox"/>	KEGG_PATHWAY	Influenza A	RT		21	2.0	9.4E-4	1.5E-2
<input type="checkbox"/>	KEGG_PATHWAY	Cell adhesion molecules (CAMs)	RT		20	1.9	2.4E-3	3.1E-2
<input type="checkbox"/>	KEGG_PATHWAY	Rheumatoid arthritis	RT		19	1.8	1.2E-6	7.9E-5
<input type="checkbox"/>	KEGG_PATHWAY	Leishmaniasis	RT		18	1.7	2.0E-7	1.7E-5
<input type="checkbox"/>	KEGG_PATHWAY	Chagas disease (American trypanosomiasis)	RT		18	1.7	5.7E-5	1.5E-3
<input type="checkbox"/>	KEGG_PATHWAY	Pertussis	RT		17	1.6	1.3E-6	6.9E-5
<input type="checkbox"/>	KEGG_PATHWAY	Toxoplasmosis	RT		17	1.6	9.7E-4	1.4E-2
<input type="checkbox"/>	KEGG_PATHWAY	Transcriptional misregulation in cancer	RT		17	1.6	1.5E-2	1.2E-1
<input type="checkbox"/>	KEGG_PATHWAY	Staphylococcus aureus infection	RT		16	1.5	1.0E-7	1.3E-5
<input type="checkbox"/>	KEGG_PATHWAY	TNF signaling pathway	RT		16	1.5	7.7E-4	1.3E-2
<input type="checkbox"/>	KEGG_PATHWAY	Malaria	RT		15	1.4	2.2E-6	7.3E-5
<input type="checkbox"/>	KEGG_PATHWAY	Measles	RT		15	1.4	1.5E-2	1.2E-1
<input type="checkbox"/>	KEGG_PATHWAY	NF-kappa B signaling pathway	RT		14	1.3	9.7E-4	1.5E-2
<input type="checkbox"/>	KEGG_PATHWAY	Leukocyte transendothelial migration	RT		14	1.3	1.3E-2	1.2E-1
<input type="checkbox"/>	KEGG_PATHWAY	Systemic lupus erythematosus	RT		14	1.3	2.7E-2	1.9E-1
<input type="checkbox"/>	KEGG_PATHWAY	Salmonella infection	RT		13	1.2	1.6E-3	2.2E-2
<input type="checkbox"/>	KEGG_PATHWAY	Cell cycle	RT		13	1.2	4.3E-2	2.8E-1
<input type="checkbox"/>	KEGG_PATHWAY	Legionellosis	RT		12	1.1	2.1E-4	4.9E-3
<input type="checkbox"/>	KEGG_PATHWAY	Inflammatory bowel disease (IBD)	RT		12	1.1	6.7E-4	1.3E-2
<input type="checkbox"/>	KEGG_PATHWAY	Natural killer cell mediated cytotoxicity	RT		12	1.1	1.7E-2	1.3E-1
<input type="checkbox"/>	KEGG_PATHWAY	Axon guidance	RT		12	1.1	8.8E-2	4.3E-1
<input type="checkbox"/>	KEGG_PATHWAY	Cytosolic DNA-sensing pathway	RT		11	1.0	7.6E-4	1.4E-2
<input type="checkbox"/>	KEGG_PATHWAY	Complement and coagulation cascades	RT		11	1.0	5.3E-3	6.1E-2
<input type="checkbox"/>	KEGG_PATHWAY	Hematopoietic cell lineage	RT		11	1.0	1.2E-2	1.2E-1
<input type="checkbox"/>	KEGG_PATHWAY	Viral myocarditis	RT		11	1.0	2.4E-2	1.8E-1
<input type="checkbox"/>	KEGG_PATHWAY	Antigen processing and presentation	RT		11	1.0	3.7E-2	2.5E-1
<input type="checkbox"/>	KEGG_PATHWAY	Toll-like receptor signaling pathway	RT		11	1.0	3.7E-2	2.5E-1
<input type="checkbox"/>	KEGG_PATHWAY	HIF-1 signaling pathway	RT		11	1.0	5.6E-2	3.3E-1
<input type="checkbox"/>	KEGG_PATHWAY	Adherens junction	RT		9	0.8	4.4E-2	2.8E-1
<input type="checkbox"/>	KEGG_PATHWAY	PPAR signaling pathway	RT		9	0.8	5.7E-2	3.3E-1
<input type="checkbox"/>	KEGG_PATHWAY	Intestinal immune network for IgA production	RT		8	0.7	1.3E-2	1.2E-1
<input type="checkbox"/>	KEGG_PATHWAY	B cell receptor signaling pathway	RT		8	0.7	8.0E-2	4.0E-1
<input type="checkbox"/>	KEGG_PATHWAY	Asthma	RT		7	0.7	5.7E-3	6.3E-2
<input type="checkbox"/>	KEGG_PATHWAY	Fatty acid metabolism	RT		7	0.7	7.1E-2	3.8E-1
<input type="checkbox"/>	KEGG_PATHWAY	Mineral absorption	RT		6	0.6	7.0E-2	3.8E-1
<input type="checkbox"/>	KEGG_PATHWAY	Biosynthesis of unsaturated fatty acids	RT		5	0.5	7.0E-2	3.8E-1

KEGG pathway term

Cell adhesion molecules

Cytokine-receptor interaction

HIF-1 signaling

Leukocyte transendothelial migration

MAPK signaling

Natural killer cell mediated cytotoxicity

NF-kappaB signaling

Phagosome

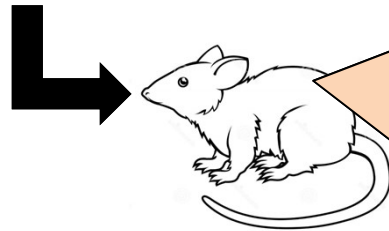
TNF signaling

Toll-like receptor signaling

免疫応答や炎症応答に
関与したシグナル経路

結論

MeHg



Death of
middle-size neuron

Degradation of
sensory axon

メチル水銀曝露ラットにおいて:

- 感覚神経線維特異的な脱落
- 中型神経細胞の脱落
- マクロファージの浸潤と
- その活性化・増加
- 痛覚特異的鈍麻と回復

が観察された。

DRG

Invasion of
macrophag
and
their
activation

