

SUPPORTING INFORMATION

**Chronic exposure of adult rats to low doses of
methyl-mercury induced a state of metabolic
deficit in the somatosensory cortex**

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Table S1. Minimum technical variabilities on relative protein quantification using ICPL labeling kit coupled with either ESI-Ion-Trap MS or MALDI-TOF/TOF MS. Minimum technical variability was calculated according to methodologies suggested by manufacturer of the ICPL kit (Bruker, Germany). Brief procedure was described in the main text. The determination was performed in triplicates and the significant threshold for differentially expressed proteins was fixed at the 2 standard deviations values from the mean of the ratio calculated. Hence, a protein with either a differential decrease/increase of at least 32% of expression in the ESI-ion-trap MS/MS system or a differential increase/decrease of at least 22% of expression in the MALDI-TOF/TOF system was taken as significantly differentially expressed.

Table S2. Amount of total mercury (tHg) found in different parts of the rat brains harvested after feeding these rats with MeHg for 4, 8 and 12 weeks. The minimum values were usually higher than 500 ppb. Values of the Sham control group as well as vehicle group are also presented. These values were always lowered than 50 ppb.

Figure S1. Results of Western blotting validation of selected proteins of interest identified in the proteomic investigation part of this study. Ten differentially expressed proteins were chosen for the validations. Expression levels of histone H3 were used to normalize protein loading for all samples. Five somatosensory cortices harvested from rats in the MeHg and Vehicle groups were used. Results shown left to the red line were from rats with CELD of MeHg while results right to the red line were from that of control rats.

Table S3. Pathway analysis of the differentially expressed proteins using Gene Orthology. Metabolic processes in the somatosensory cortex found to be significantly affected by chronic intake of MeHg administration. These processes included protein metabolism, morphogenesis of cellular components, carbohydrate metabolism, signal transduction and neurotransmission, generation of precursor metabolites and energy, cellular amino acid and derivative metabolism, sensory perception and ion transport.

Table S4. Identities of up-regulated proteins found in the somatosensory cortices of

rats fed with 40 µg/kg body weight/day for 12 weeks. Ten proteins of 8 different metabolic processes were affected. Myelin-associated oligodendrocyte basic protein and myelin basic protein isoform 4 are the only 2 proteins that are related to the same category of cellular component morphogenesis. The remaining 8 proteins are not directly related to each other.

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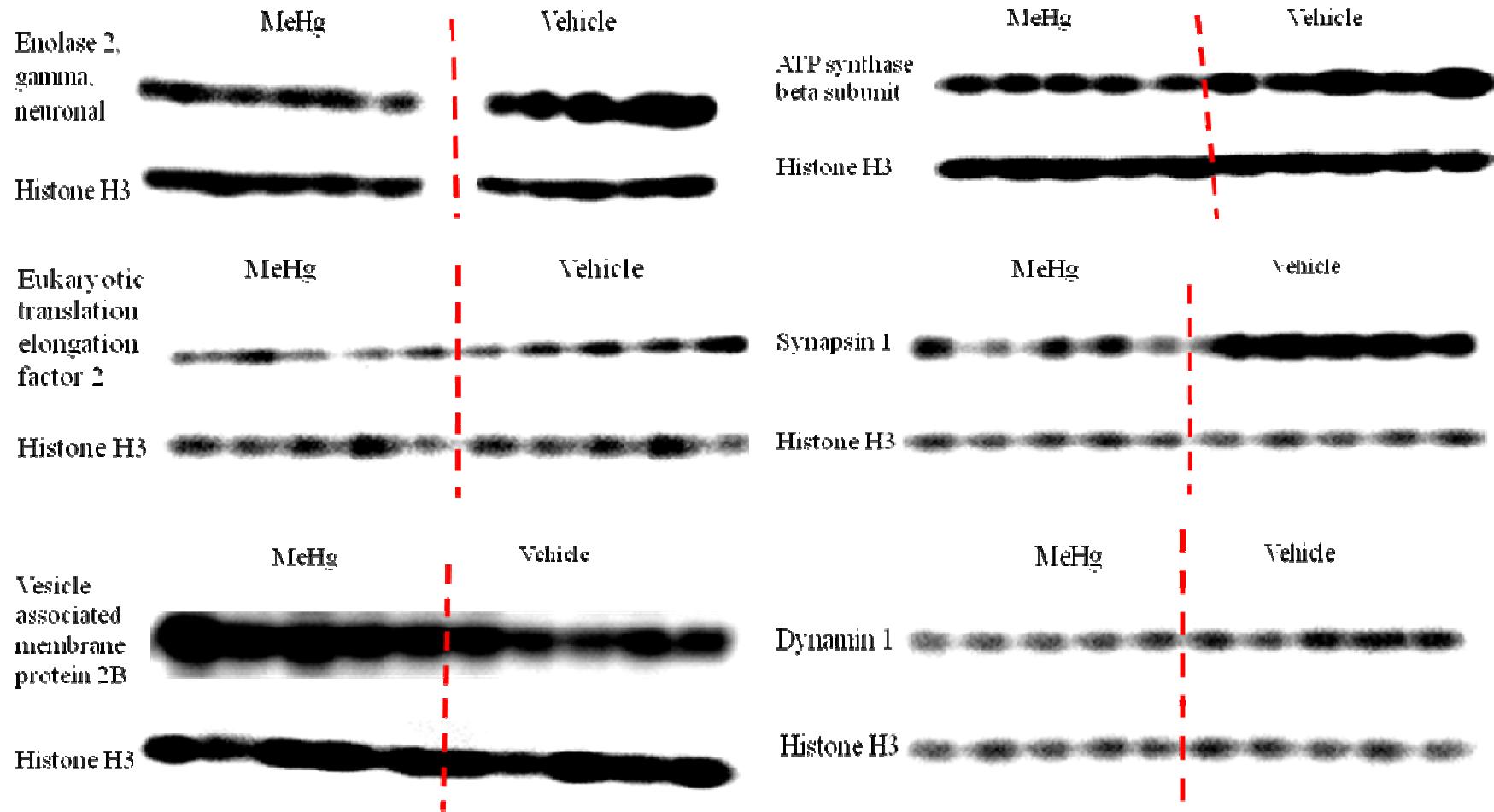
	Mean ± SD*
LC ESI- Ion-Trap MS/MS	0.96 ± 0.16
LC MALDI-TOF/TOF MS	0.86 ± 0.11

* Mean was defined as the mean of the ratio of the peak areas of the peptides labeled with either heavy or light ICPL.

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		tHg in ppb, ng / g of tissue, Mean \pm SD		
		4 weeks	8 weeks	12 weeks
Sham control	Motor cortex	12.6 \pm 4.8	15.3 \pm 0.6	18.9 \pm 4.9
	Somatosensory cortex	12.7 \pm 1.8	12.9 \pm 0.8	16.8 \pm 3.1
	Visual cortex	12.6 \pm 0.8	22.6 \pm 1.8	27.1 \pm 7.0
	Cerebellum	13.5 \pm 2.0	32.9 \pm 4.5	44.9 \pm 7.8
	Motor cortex	6.1 \pm 0.6	8.3 \pm 2.6	12.9 \pm 5.7
	Somatosensory cortex	7.5 \pm 0.3	11.4 \pm 3.5	17.8 \pm 5.6
	Visual cortex	7.6 \pm 1.3	13.4 \pm 5.6	19.0 \pm 4.4
	Cerebellum	7.6 \pm 0.6	22.1 \pm 6.5	33.2 \pm 8.3
	Motor cortex	527.2 \pm 59.0	648.3 \pm 79.2	1002.2 \pm 79.1
Vehicle	Somatosensory cortex	590.3 \pm 46.4	719.6 \pm 67.9	901.6 \pm 54.1
	Visual cortex	607.1 \pm 118.0	808.4 \pm 50.5	1189.0 \pm 89.7
	Cerebellum	622.5 \pm 27.6	839.9 \pm 48.7	1436.1 \pm 133.8

Each group at each time point contained 12 animals.



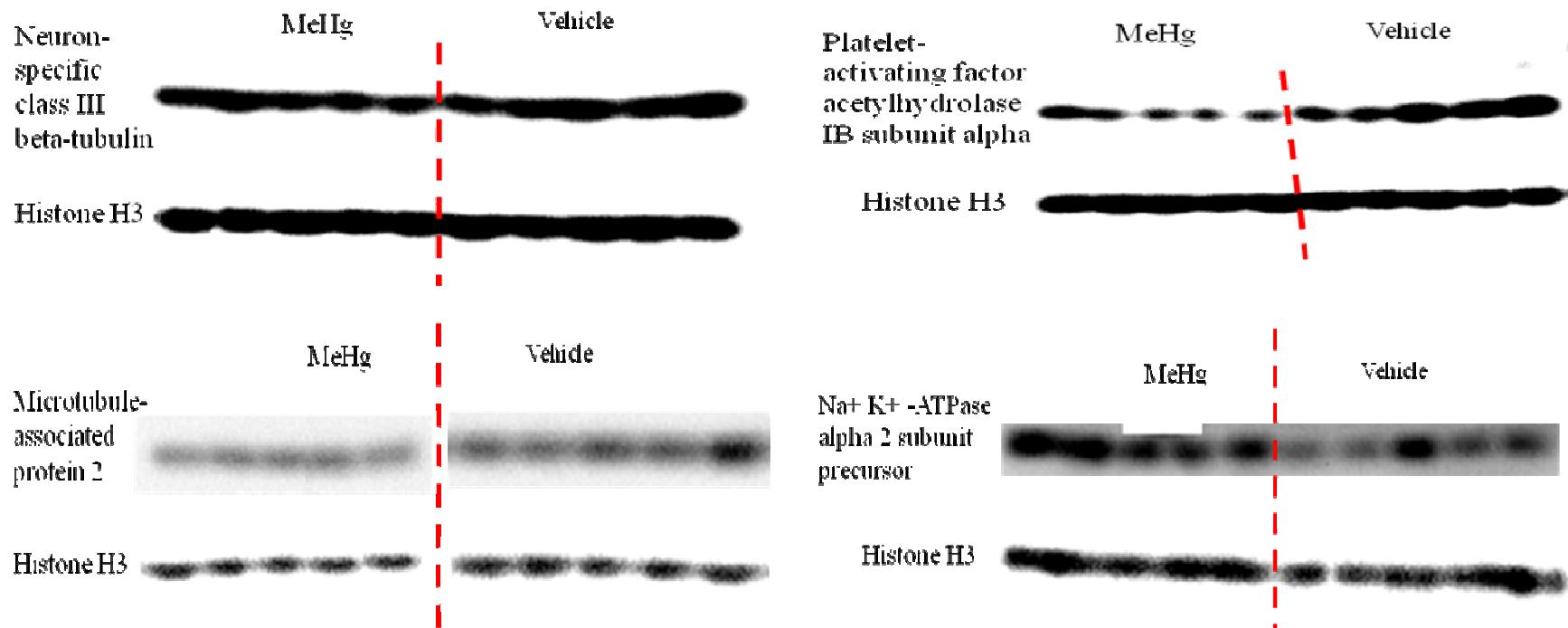


Figure S1. Results of Western blotting validation of selected proteins of interest identified in the proteomic investigation part of this study. Ten differentially expressed proteins were chosen for the validations. Expression levels of histone H3 were used to normalize protein loading for all samples. Five somatosensory cortices harvested from rats in the MeHg and Vehicle groups were used. Results shown left to the red line were from rats with CELD of MeHg while results right to the red line were from that of control rats.

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Protein metabolic process, 19%

Protein metabolic process, GO: 0019538

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
Mitochondrial isoleucine tRNA synthetase [Rattus norvegicus]	gil206597496	LC MALDI	46.2	47.9	9.7	10.1	3	↓0.43 ± 0.19	↓ 57 ± 19

Translation, GO: 0006412

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
Eukaryotic translation elongation	gil8393296	LC ESI	447.3	96.2	6.4	17.1	21	↓0.63 ± 0.08	↓ 37 ± 8
Factor 2 [Rattus norvegicus]		LC MALDI	267.9	96.2	6.4	11.4	14	↓0.48 ± 0.04	↓ 52 ± 4
Ribosomal protein L9 [Rattus norvegicus]	gil38051904	LC ESI	177.5	22	10.6	33.3	7	↓0.63 ± 0.14	↓ 37 ± 14
Tu translation elongation factor, mitochondrial precursor [Rattus norvegicus]	gil157820845	LC MALDI	100.1	22	10.6	23.4	4	↓0.58 ± 0.08	↓ 42 ± 8
		LC ESI	190.3	49.9	7.9	17.7	13	↓0.61 ± 0.04	↓ 39 ± 4
		LC MALDI	81.6	49.9	7.9	7.5	3	↓0.47 ± 0.18	↓ 53 ± 18

Ribosomal protein L5 [Rattus norvegicus]	gil206734	LC ESI	155.9	34.5	10.2	15.9	5	$\downarrow 0.47 \pm 0.33$	$\downarrow 53 \pm 22$
Ribosomal protein L7, partial [Rattus norvegicus]	gil206736	LC ESI	296.7	29.3	11.2	29.8	9	$\downarrow 0.39 \pm 0.23$	$\downarrow 61 \pm 23$
40S Ribosomal protein S18 [Rattus norvegicus]	gil47087103	LC ESI	148	17.7	11.5	26.3	4	$\uparrow 1.30 \pm 0.07$	$\uparrow 30 \pm 7$

Protein complex assembly, GO: 0006461 & Protein folding, GO: 0006457

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
Heat shock protein 105kDa [Rattus norvegicus]	gil58865372	LC ESI	359.2	97.3	5.3	15.2	13	$\downarrow 0.53 \pm 0.09$	$\downarrow 47 \pm 9$
		LC MALDI	177	97.3	5.3	16.1	7	$\downarrow 0.50 \pm 0.07$	$\downarrow 50 \pm 7$
Heat shock protein HSP 90-beta [Rattus norvegicus]	gil148747365	LC ESI	890.5	83.6	4.8	35.2	34	$\downarrow 0.63 \pm 0.03$	$\downarrow 37 \pm 3$
		LC MALDI	439.5	83.6	4.8	19.9	21	$\downarrow 0.53 \pm 0.07$	$\downarrow 47 \pm 7$
Heat shock protein 4 [Rattus norvegicus]	gil24025637	LC ESI	632	94.8	5	21.5	20	$\downarrow 0.54 \pm 0.03$	$\downarrow 46 \pm 3$
		LC MALDI	323.2	94.8	5	12.3	13	$\downarrow 0.46 \pm 0.02$	$\downarrow 54 \pm 2$
Heat shock protein 8 [Rattus norvegicus]	gil13242237	LC ESI	599.2	71.1	5.2	28.9	27	$\downarrow 0.55 \pm 0.03$	$\downarrow 45 \pm 3$
		LC MALDI	777.5	71.1	5.2	30.2	24	$\downarrow 0.54 \pm 0.06$	$\downarrow 46 \pm 6$
Heat shock protein 90, alpha (cytosolic), class A member 1 [Rattus norvegicus]	gil28467005	LC ESI	1045.6	85.2	4.8	44.1	43	$\downarrow 0.58 \pm 0.03$	$\downarrow 42 \pm 3$
		LC MALDI	763.7	85.2	4.8	28.8	33	$\downarrow 0.53 \pm 0.05$	$\downarrow 47 \pm 5$

Heat shock protein 2 [Rattus norvegicus]	gil11177910	LC ESI	469.2	69.9	5.4	14.8	27	$\downarrow 0.60 \pm 0.15$	$\downarrow 40 \pm 15$
		LC MALDI	284.7	69.8	5.3	10.3	10	$\downarrow 0.47 \pm 0.03$	$\downarrow 53 \pm 3$
Heat shock protein 5 precursor [Rattus norvegicus]	gil25742763	LC MALDI	82.9	72.5	4.9	9.8	3	$\downarrow 0.55 \pm 0.05$	$\downarrow 45 \pm 5$
Translocase of outer mitochondrial membrane 70 homolog A [Rattus norvegicus]	gil47058988	LC MALDI	45.3	68.1	8.4	9.3	3	$\downarrow 0.46 \pm 0.10$	$\downarrow 54 \pm 10$
DNA K-type molecular chaperone hsp72-ps1 - rat	gil347019	LC ESI	584.9	71.1	5.3	28.9	21	$\downarrow 0.53 \pm 0.02$	$\downarrow 47 \pm 2$
Endoplasmin precursor [Rattus norvegicus]	gil210032365	LC ESI	118.3	74.4	4.9	9.3	4	$\downarrow 0.50 \pm 0.25$	$\downarrow 50 \pm 25$

Proteolysis, GO: 0006508

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
Ubiquinol cytochrome c reductase core protein 2 precursor [Rattus norvegicus]	gil55741544	LC ESI	484.8	48.4	9.6	36.9	23	$\downarrow 0.55 \pm 0.06$	$\downarrow 45 \pm 6$
		LC MALDI	311.2	48.4	9.6	25.2	14	$\downarrow 0.50 \pm 0.12$	$\downarrow 50 \pm 12$
Ubiquitin specific protease 7 (herpes virus-associated) [Rattus norvegicus]	gil149042601	LC MALDI	55.9	116.5	5.3	3	3	$\downarrow 0.24 \pm 0.03$	$\downarrow 76 \pm 3$

Proteasome (prosome, macropain) subunit, alpha type 3 [Rattus norvegicus] gil8394066 LC ESI 139.8 28.6 5.2 22 7 ↓0.55 ± 0.03 ↓ 45 ± 3

Cellular component morphogenesis, 17%

Cellular component morphogenesis, GO: 0032989

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression	
								Fold change ^	% §
Actin related protein 2/3 complex, subunit 2 [Rattus norvegicus]	gil157817229	LC ESI	235.4	34.4	7	23	7	↓0.64 ± 0.05	↓ 36 ± 5
		LC MALDI	175.4	34.4	7	20.7	7	↓0.54 ± 0.15	↓ 46 ± 15
Actin, beta-like 2 [Rattus norvegicus]	gil157823033	LC ESI	642.1	42.3	5.2	35.1	27	↓0.61 ± 0.06	↓ 39 ± 6
		LC MALDI	298.4	42.3	5.2	27.7	17	↓0.52 ± 0.02	↓ 48 ± 2
Internexin, alpha [Rattus norvegicus]	gil149040328	LC ESI	616.3	56.4	5	35.6	30	↓0.57 ± 0.10	↓ 43 ± 10
		LC MALDI	362.7	56.4	5	22.1	12	↓0.44 ± 0.02	↓ 56 ± 2
Neurofilament, heavy polypeptide [Rattus norvegicus]	gil158341642	LC ESI	402.6	114.6	5.6	10.2	18	↓0.58 ± 0.12	↓ 42 ± 12
		LC MALDI	244.9	114.6	5.6	10	10	↓0.50 ± 0.07	↓ 50 ± 7
Neurofilament, light polypeptide [Rattus norvegicus]	gil13929098	LC ESI	509.2	61.4	4.5	26	23	↓0.56 ± 0.03	↓ 44 ± 3
		LC MALDI	306.5	61.4	4.5	17.5	11	↓0.40 ± 0.01	↓ 60 ± 1
Spectrin-like protein GTRAP41 [Rattus norvegicus]	gil11066461	LC ESI	740.3	272.1	5.5	11.9	19	↓0.54 ± 0.08	↓ 46 ± 8
		LC MALDI	374.1	272.1	5.5	7.7	22	↓0.56 ± 0.05	↓ 44 ± 5
Tubulin, alpha 1A [Rattus	gil38328248	LC ESI	977.5	50.8	4.8	51.4	21	↓0.57 ± 0.03	↓ 43 ± 3

norvegicus]		LC MALDI	1750.2	50.8	4.8	75.2	24	$\downarrow 0.53 \pm 0.06$	$\downarrow 47 \pm 6$
Tubulin, beta 6 [Rattus	gil71043680	LC ESI	706.5	50.5	4.6	31.5	28	$\downarrow 0.54 \pm 0.07$	$\downarrow 46 \pm 7$
norvegicus]		LC MALDI	597.6	50.5	4.6	27.1	16	$\downarrow 0.47 \pm 0.06$	$\downarrow 53 \pm 6$
270 kDa Ankyrin G isoform	gil3885972	LC MALDI	92.9	285.7	8.6	3.7	3	$\downarrow 0.38 \pm 0.06$	$\downarrow 62 \pm 6$
[Rattus norvegicus]									
Microtubule-associated protein 1B	gil158749620	LC MALDI	279.7	270.7	4.6	5.9	15	$\downarrow 0.54 \pm 0.04$	$\downarrow 46 \pm 4$
[Rattus norvegicus]									
Myosin, heavy chain 10,	gil13928704	LC MALDI	151.3	229.8	5.4	5.1	14	$\downarrow 0.46 \pm 0.05$	$\downarrow 54 \pm 5$
non-muscle [Rattus norvegicus]									
Neuron-specific class III	gil20799322	LC MALDI	787.9	50.7	4.7	39.6	38	$\downarrow 0.44 \pm 0.05$	$\downarrow 56 \pm 5$
beta-tubulin [Rattus norvegicus]									
Spectrin alpha chain, brain [Rattus	gil31543764	LC MALDI	1022.8	283	5.1	15.7	41	$\downarrow 0.44 \pm 0.03$	$\downarrow 56 \pm 3$
norvegicus]									
Myelin-associated oligo-	gil6981216	LC MALDI	86.7	19.3	12.3	26	3	$\uparrow 3.70 \pm 0.37$	$\uparrow 270 \pm 37$
dendrocyte basic protein [Rattus									
norvegicus]									
Microtubule-associated protein 2,	gil149016020	LC ESI	684.1	198.7	4.6	14.4	37	$\downarrow 0.58 \pm 0.14$	$\downarrow 42 \pm 14$
isoform CRA_b [Rattus									
norvegicus]									
NF-M protein [Rattus norvegicus]	gil205688	LC ESI	516.2	95.7	4.6	19.6	23	$\downarrow 0.53 \pm 0.13$	$\downarrow 47 \pm 13$
Myelin basic protein isoform 4	gil70166270	LC ESI	93.2	17.2	11.3	36.7	4	$\uparrow 1.65 \pm 0.28$	$\uparrow 65 \pm 28$
[Rattus norvegicus]									

Sirtuin (silent mating type information regulation 2 homolog) 2 [Rattus norvegicus]	gil56605812	LC ESI	332	39.9	6.8	31.4	10	$\downarrow 0.64 \pm 0.05$	$\downarrow 36 \pm 5$
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Carbohydrate metabolic process, 16%

Glycolysis, GO:0006096

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression	
								Fold change ^	% §
Enolase 2, gamma, neuronal [Rattus norvegicus]	gil26023949	LC ESI	627.2	47.5	5	44.5	33	$\downarrow 0.55 \pm 0.21$	$\downarrow 45 \pm 21$
		LC MALDI	293	47.5	5	24.2	15	$\downarrow 0.59 \pm 0.15$	$\downarrow 41 \pm 15$
M2 pyruvate kinase [Rattus norvegicus]	gil206205	LC ESI	1066.8	58.3	8	51.2	38	$\downarrow 0.61 \pm 0.02$	$\downarrow 39 \pm 2$
		LC MALDI	613.8	58.3	8	30.3	24	$\downarrow 0.52 \pm 0.10$	$\downarrow 48 \pm 10$
Phosphofructokinase, muscle [Rattus norvegicus]	gil62825891	LC ESI	508.3	86.1	9	19	31	$\downarrow 0.41 \pm 0.08$	$\downarrow 59 \pm 8$
		LC MALDI	543.6	86.1	9	21	24	$\downarrow 0.46 \pm 0.05$	$\downarrow 54 \pm 5$
Hexokinase 1 [Rattus norvegicus]	gil6981022	LC MALDI	1475.9	103.5	6	42.5	55	$\downarrow 0.55 \pm 0.06$	$\downarrow 45 \pm 6$
Aldolase A [Rattus norvegicus]	gil202837	LC MALDI	310.3	39.7	9	26.4	12	$\downarrow 0.43 \pm 0.11$	$\downarrow 57 \pm 11$
Fructose-bisphosphate aldolase C [Rattus norvegicus]	gil6978489	LC ESI	582	39.7	7	35.8	17	$\uparrow 1.53 \pm 0.35$	$\uparrow 53 \pm 35$

Dihydrolipoamide S-acetyltransferase [Rattus norvegicus]	gil78365255	LC ESI	314.3	67.6	10	15.3	10	$\downarrow 0.59 \pm 0.08$	$\downarrow 41 \pm 8$
Tpi1 protein, triosephosphate isomerase [Rattus norvegicus]	gil38512111	LC ESI	304.7	27.2	8	38.3	12	$\downarrow 0.63 \pm 0.40$	$\downarrow 37 \pm 40$
Lactate dehydrogenase A [Rattus norvegicus]	gil8393706	LC ESI	273.4	36.7	9	16.9	11	$\downarrow 0.50 \pm 0.07$	$\downarrow 50 \pm 7$

Tricarboxylic acid cycle, GO: 0006099

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
Aconitase 2, mitochondrial precursor [Rattus norvegicus]	gil40538860	LC ESI	1216	86.1	9	38.2	44	$\downarrow 0.61 \pm 0.03$	$\downarrow 39 \pm 3$
		LC MALDI	987.1	86.1	9	36.3	42	$\downarrow 0.54 \pm 0.05$	$\downarrow 46 \pm 5$
Isocitrate dehydrogenase 3, beta subunit precursor [Rattus norvegicus]	gil55926203	LC ESI	522.6	42.6	10	28.6	19	$\downarrow 0.57 \pm 0.04$	$\downarrow 43 \pm 4$
		LC MALDI	85.5	42.6	10	16.1	3	$\downarrow 0.39 \pm 0.16$	$\downarrow 61 \pm 16$
Oxoglutarate dehydrogenase-like [Rattus norvegicus]	gil157819765	LC ESI	376.5	117.5	6	11.3	12	$\downarrow 0.66 \pm 0.10$	$\downarrow 34 \pm 10$
		LC MALDI	96.3	117.5	6	4.5	3	$\downarrow 0.43 \pm 0.04$	$\downarrow 57 \pm 4$
Pyruvate carboxylase precursor [Rattus norvegicus]	gil31543464	LC MALDI	243.3	130.4	6	11.8	16	$\downarrow 0.42 \pm 0.05$	$\downarrow 58 \pm 5$

Malic enzyme 3, NADP(+) -dependent, mitochondrial (predicted), isoform CRA_b [Rattus norvegicus]	gil149069022	LC MALDI	68.4	35.2	9	15.3	3	$\downarrow 0.53 \pm 0.07$	$\downarrow 47 \pm 7$
malate dehydrogenase 1, NAD (soluble) [Rattus norvegicus]	gil15100179	LC ESI	434.5	36.6	6	24.3	23	$\downarrow 0.62 \pm 0.05$	$\downarrow 38 \pm 5$

Pentose-phosphate shunt, GO: 0006098

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression	
								Fold change ^	% §
Transketolase, isoform CRA_a [Rattus norvegicus]	gil149034221	LC ESI	372	71.9	9	14.5	7	$\downarrow 0.58 \pm 0.03$	$\downarrow 42 \pm 3$
		LC MALDI	259	71.9	9	11.9	8	$\downarrow 0.39 \pm 0.08$	$\downarrow 61 \pm 8$

Glycogen metabolic process, GO: 0005977

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression	
								Fold change ^	% §
Muscle glycogen phosphorylase [Rattus norvegicus]	gil158138498	LC ESI	337	97.7	7	15.6	11	$\downarrow 0.64 \pm 0.08$	$\downarrow 36 \pm 8$
		LC MALDI	205.7	97.7	7	10.9	9	$\downarrow 0.47 \pm 0.06$	$\downarrow 53 \pm 6$

Signal transduction, 12%

Cell surface receptor linked signal transduction, GO: 0007166

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
Tyrosine 3-monooxygenase / Tryptophan 5-monooxygenase activation protein, gamma polypeptide [Rattus norvegicus]	gil9507245	LC ESI LC MALDI	352.5 151.3	28.5 28.5	4.7 4.7	32.8 13.8	13 5	↓0.63 ± 0.10 ↓0.48 ± 0.07	↓ 37 ± 10 ↓ 52 ± 7
Ankyrin binding cell adhesion molecule neurofascin [Rattus norvegicus]	gil1842427	LC MALDI	132	132	6.2	9.8	4	↑ 1.65 ± 0.16	↑ 65 ± 16
Annexin A6 [Rattus norvegicus]	gil130502086	LC MALDI	81.5	76.1	5.3	7.9	3	↓0.29 ± 0.08	↓ 71 ± 8

Intercellular signaling cascade, GO: 0007242

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
CAP, adenylate cyclase-associated protein, 2 [Rattus norvegicus]	gil16758742	LC ESI LC MALDI	156.5 80.2	53.2 53.2	6.8 6.8	11.7 6.9	5 3	↓0.68 ± 0.11 ↓0.42 ± 0.02	↓ 32 ± 11 ↓ 58 ± 2
ras-Related C3 botulinum toxin substrate 1, isoform CRA_b [Rattus norvegicus]	gil149034945	LC MALDI	116	21.8	9.7	15.6	4	↑1.94 ± 0.02	↑ 94 ± 2

Synaptic ras GTPase-activating protein p135 SynGAP [Rattus norvegicus]	gil2935448	LC MALDI	391.7	144	9.8	17.3	15	$\downarrow 0.52 \pm 0.01$	$\downarrow 48 \pm 1$
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Ca++-mediated signaling, GO: 0019722

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression	
								Fold change ^	% §
Serine/threonine-protein kinase 2A 65 kDa regulatory subunit A alpha isoform [Rattus norvegicus]	gil34854430	LC ESI	471.1	66.1	4.9	22.8	11	$\downarrow 0.68 \pm 0.14$	$\downarrow 32 \pm 14$
Calcium/calmodulin-dependent protein kinase II alpha [Rattus norvegicus]	gil6978593	LC ESI	178.8	54.7	6.7	12.1	7	$\downarrow 0.55 \pm 0.04$	$\downarrow 45 \pm 4$
Calmodulin dependent protein kinase II	gil225775	LC MALDI	522	54.7	6.7	29.3	26	$\downarrow 0.55 \pm 0.15$	$\downarrow 45 \pm 15$
Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2 [Rattus norvegicus]	gil29789261	LC ESI	370.9	38	5.6	47.9	18	$\downarrow 0.59 \pm 0.05$	$\downarrow 41 \pm 5$
Protein kinase C, beta [Rattus norvegicus]	gil76880457	LC MALDI	553.9	38	5.6	50.6	23	$\downarrow 0.51 \pm 0.05$	$\downarrow 49 \pm 5$
Protein kinase C gamma	gil6981400	LC ESI	86.9	77.9	6.5	6	3	$\downarrow 0.46 \pm 0.06$	$\downarrow 54 \pm 6$
			140.1	78.1	6.6	9.7	6	$\downarrow 0.49 \pm 0.06$	$\downarrow 51 \pm 6$

type [Rattus norvegicus]	LC MALDI	119.7	79.6	7.9	7.2	5	$\downarrow 0.54 \pm 0.04$	$\downarrow 46 \pm 4$
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Generation of precursor metabolites and energy, 11%

Respiratory electron transport chain, GO: 0022904

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^ % §
ATP synthase alpha subunit precursor (EC 3.6.1.3), partial [Rattus norvegicus]	gil203055	LC ESI LC MALDI	1094.4 1217.2	58.9 58.9	10 10	56.2 40.9	31 28	$\downarrow 0.61 \pm 0.02$ $\downarrow 0.50 \pm 0.08$ $\downarrow 39 \pm 2$ $\downarrow 50 \pm 8$
ATP synthase beta subunit [Rattus norvegicus]	gil1374715	LC ESI LC MALDI	929.8 471.1	51.2 51.2	5 5	48.6 36	35 23	$\downarrow 0.62 \pm 0.05$ $\downarrow 0.43 \pm 0.09$ $\downarrow 38 \pm 5$ $\downarrow 57 \pm 9$
ATPase, H ⁺ transporting, lysosomal V1 subunit A [Rattus norvegicus]	gil157819953	LC ESI LC MALDI	757.5 555	68.6 68.6	5 5	28.4 31.9	35 26	$\downarrow 0.58 \pm 0.01$ $\downarrow 0.50 \pm 0.04$ $\downarrow 42 \pm 1$ $\downarrow 50 \pm 4$
NADH dehydrogenase (ubiquinone) Fe-S protein 1, 75kDa precursor [Rattus norvegicus]	gil53850628	LC ESI LC MALDI	927.4 608.5	80.3 80.3	6 6	29.6 23.2	33 24	$\downarrow 0.54 \pm 0.02$ $\downarrow 0.49 \pm 0.02$ $\downarrow 46 \pm 2$ $\downarrow 51 \pm 2$
Succinate dehydrogenase complex, subunit A, flavoprotein precursor [Rattus norvegicus]	gil18426858	LC MALDI	252.6	72.6	7	18.9	11	$\downarrow 0.49 \pm 0.03$ $\downarrow 51 \pm 3$
F1-ATPase beta subunit, partial [Rattus norvegicus]	gil203033	LC MALDI	455.4	38.7	5	38.3	17	$\downarrow 0.43 \pm 0.09$ $\downarrow 57 \pm 9$

ATP synthase, H ⁺ transporting, mitochondrial F0 complex, subunit B1 precursor [Rattus norvegicus]	gil19705465	LC MALDI	169.2	29	10	14.8	4	↓0.52 ± 0.06	↓ 48 ± 6
Glycerol-3-phosphate dehydrogenase 2, mitochondrial precursor [Rattus norvegicus]	gil6980978	LC MALDI	47.4	81.5	6	6.1	2	↓0.33 ± 0.07	↓ 77 ± 7
Hydroxyacylglutathione hydrolase [Rattus norvegicus]	gil15375320	LC MALDI	48.8	29.2	7	10.4	2	↓0.49 ± 0.07	↓ 51 ± 7
Cytochrome c, somatic [Rattus norvegicus]	gil6978725	LC ESI	88	11.7	10	13.3	8	↓0.35 ± 0.07	↓ 65 ± 7
Inner membrane protein, mitochondrial, isoform CRA_a [Rattus norvegicus]	gil149036390	LC ESI	287.1	86.2	6	13.5	10	↓0.51 ± 0.06	↓ 49 ± 6

Phosphocreatine biosynthetic process, GO: 0046314

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
Creatine kinase, mitochondrial 1, ubiquitous precursor [Rattus norvegicus]	gil60678254	LC ESI	511.6	47.3	9	34.9	16	↓0.57 ± 0.04	↓ 43 ± 4

Neurotransmission related process, 9%

Exocytosis, GO: 0006887

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡		Change in expression	
							Fold change ^	% §		
Valosin-containing protein [Rattus norvegicus]	gil17865351	LC ESI LC MALDI	727.1 197.2	90 90	5 5	28.9 21.1	20 12	↓0.58 ± 0.06 ↓0.54 ± 0.02	↓ 42 ± 6 ↓ 46 ± 2	
Vesicle associated membrane protein 2B [Rattus norvegicus]	gil4894188	LC ESI LC MALDI	182.8 120.2	14.6 14.6	5.3 5.3	23 23	5 4	↑3.26 ± 0.55 ↑4.73 ± 0.80	↑ 226 ± 55 ↑ 373 ± 80	
SNIP-a [Rattus norvegicus], SNAP-25 interacting protein	gil6693834	LC MALDI	121.9	127.2	9.9	15	7	↓0.49 ± 0.03	↓ 51 ± 3	

Endocytosis, GO: 0006897

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡		Change in expression	
							Fold change ^	% §		
Dynamin 1 [Rattus norvegicus]	gil18093102	LC ESI LC MALDI	1253.7 787.5	96.2 96.2	6.3 6.3	42 29.1	35 22	↓0.60 ± 0.02 ↓0.52 ± 0.03	↓ 40 ± 2 ↓ 48 ± 3	
Coronin, actin binding protein 1A [Rattus norvegicus]	gil18426834	LC MALDI	111	51.7	6	13.7	5	↓0.50 ± 0.11	↓ 50 ± 11	

Synaptic transmission, GO: 0007268

Protein name	NCBInr	Method of	MASCOT	MW,	pI	Sequence	Peptides	Change in expression	
	accession no.	identification #	score *	kDa		Coverage, % †	assigned ‡	Fold change ^	% §
Vesicle-fusing ATPase [Rattus norvegicus]	gi 13489067	LC ESI	883.9	80.6	6	34.6	42	↓0.64 ± 0.03	↓ 36 ± 3
		LC MALDI	541.1	80.6	6	25.3	28	↓0.54 ± 0.06	↓ 46 ± 6
Synapsin I isoform a [Rattus norvegicus]	gi 9507159	LC MALDI	568.5	74.1	10.3	29.8	22	↓0.58 ± 0.03	↓ 42 ± 3
RAB GDP dissociation inhibitor alpha [Rattus norvegicus]	gi 4585567	LC MALDI	328.6	41.1	5.2	32	10	↓0.47 ± 0.03	↓ 53 ± 3
Syntaxin binding protein 1, isoform CRA_a [Rattus norvegicus]	gi 148038988	LC ESI	818.9	69.1	6.3	40.8	67	↓0.65 ± 0.08	↓ 35 ± 8

Cellular amino acid and derivative metabolic process, 6%

Cellular amino acid and derivative metabolic process, GO: 0006519

Protein name	NCBInr	Method of	MASCOT	MW,	pI	Sequence	Peptides	Change in expression	
	accession no.	identification #	score *	kDa		Coverage, % †	assigned ‡	Fold change ^	% §
Cytosolic aspartate aminotransferase [Rattus norvegicus]	gi 220684	LC ESI	623.9	46.6	7	36.1	14	↓0.52 ± 0.07	↓ 48 ± 7
		LC MALDI	766.3	46.6	7	42.1	24	↓0.52 ± 0.04	↓ 48 ± 4

Glutamine synthetase [Rattus norvegicus]	gil204349	LC ESI LC MALDI	391.1 383.9	43 43	7 7	27.6 26.5	16 11	$\downarrow 0.37 \pm 0.13$ $\downarrow 0.37 \pm 0.09$	$\downarrow 63 \pm 13$ $\downarrow 63 \pm 9$
Transaminase, Glu oxaloacetic	gil350611	LC ESI LC MALDI	538.7 397.8	44.7 44.7	9 9	33.9 31.2	23 13	$\downarrow 0.59 \pm 0.05$ $\downarrow 0.42 \pm 0.05$	$\downarrow 41 \pm 5$ $\downarrow 58 \pm 5$
Platelet-activating factor acetylhydrolase IB subunit alpha [Rattus norvegicus]	gil13929078	LC ESI LC MALDI	112.3 59.6	47.2 47.2	7 7	10.2 7.3	4 2	$\downarrow 0.53 \pm 0.07$ $\downarrow 0.42 \pm 0.06$	$\downarrow 47 \pm 7$ $\downarrow 58 \pm 6$
Glutamate dehydrogenase 1 precursor [Rattus norvegicus]	gil6980956	LC MALDI	279.9	61.7	9	18.3	15	$\downarrow 0.52 \pm 0.07$	$\downarrow 48 \pm 7$
Phosphoserine aminotransferase [Rattus norvegicus]	gil29692074	LC ESI	266.2	40.9	9	28.1	12	$\downarrow 0.59 \pm 0.05$	$\downarrow 41 \pm 5$

Sensory perception, 3%

Sensory perception, GO: 0007600

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
WD repeat domain 1 [Rattus norvegicus]	gil62078997	LC ESI LC MALDI	295.2 179	66.8 66.8	6.2 6.2	18.5 21.6	12 14	$\downarrow 0.54 \pm 0.02$ $\downarrow 0.47 \pm 0.05$	$\downarrow 46 \pm 2$ $\downarrow 53 \pm 5$
Ca++-dependent secretion activator [Rattus norvegicus]	gil6978599	LC MALDI	51.6	147.3	5.5	2.7	2	$\downarrow 0.48 \pm 0.17$	$\downarrow 52 \pm 17$

Thiomorpholine-carboxylate dehydrogenase [Rattus norvegicus]	gil16758840	LC ESI	250.8	33.7	5.2	20.8	7	$\downarrow 0.59 \pm 0.13$	$\downarrow 41 \pm 13$
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Ion transport, 3%

Anion transport, GO: 0006820

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
Voltage dependent anion channel [Rattus norvegicus]	gil4558732	LC ESI LC MALDI	350.9 266.8	32.1 30.1	9.1 9.8	25.3 23	7 7	$\downarrow 0.56 \pm 0.05$ $\downarrow 0.40 \pm 0.13$	$\downarrow 44 \pm 5$ $\downarrow 60 \pm 13$

Cation transport, GO: 0006812

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
Potassium voltage-gated channel, shaker-related subfamily, beta member 2 [Rattus norvegicus]	gil8393646	LC MALDI	102.5	41.3	9.7	30.5	10	$\downarrow 0.43 \pm 0.04$	$\downarrow 57 \pm 4$
Na+/K+ -ATPase alpha 2 subunit precursor [Rattus norvegicus]	gil6978545	LC MALDI	107.7	113.5	5.3	6.3	3	$\uparrow 1.75 \pm 0.15$	$\uparrow 75 \pm 15$

Others, 4%

Nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, GO: 0006139

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^ % §
Heterogeneous nuclear ribonucleoprotein U [Rattus norvegicus]	gil624918	LC ESI	299	88.5	6	10.3	11	↓0.55 ± 0.02 ↓ 45 ± 2
Dihydropyrimidinase-related protein 2 [Rattus norvegicus]	gil157786744	LC MALDI	327.8	88.5	6	20.6	14	↓0.55 ± 0.13 ↓ 45 ± 13
			134.6	62.6	6	10.7	4	↑4.03 ± 2.27 ↑ 303 ± 227

Response to stress, GO: 0006950

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^ % §
Glutathione S-transferase alpha [Rattus norvegicus]	gil7188365	LC MALDI	102.7	26	9	21.2	3	↓0.51 ± 0.09 ↓ 49 ± 9

Mitosis, GO: 0007067

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^ % §
Centromere protein F [Rattus norvegicus]	gil281371331	LC ESI	56.7	306.2	5	3.1	2	2.32 ± 0.66 ↑ 132 ± 66

#Method of identification: LC ESI: identified by nano C₁₈ liquid chromatography coupled with electrospray ionization ion-trap tandem mass spectrometry. LC MALDI: identified by nano C₁₈ liquid chromatography coupled with matrix assisted laser desorption ionization time-of-flight/time-of-flight mass spectrometry.

*MASCOT score: Sum of the ion scores of the peptides assigned to the identified proteins.

†Sequence Coverage %: Sum of the percentages of amino acid sequences covered by the peptides assigned to the identified proteins.

‡Peptide assigned: Number of peptides assigned to the identified protein with ion scores above 95% cutoff values ($p < 0.05$).

^ Fold change was the mean fold changes of the expression levels of the differentially expressed proteins followed by standard deviation. The fold change = peak area of the peptide assigned from MeHg group / peak area of the peptide assigned from vehicle group. ↑ : increased in percentage. ↓ : decreased in percentage. Six biological replicates were used.

§Percentage change in expression, % expressed as the mean of percentage change of the peak area of peptides assigned to the protein identified, ± standard deviation. ↑ : increased in percentage. ↓ : decreased in percentage. Percentage change in expression for each peptide assigned= (peak area of the peptide assigned from MeHg group – peak area of the peptide assigned from vehicle group) / peak area of the peptide assigned from vehicle group x 100%. Six biological replicates were used.

Table S4. Identities of up-regulated proteins found in the somatosensory cortices of rats fed with 40 µg/kg body weight/day for 12 weeks. Ten proteins of 8 different metabolic processes were affected. Myelin-associated oligodendrocyte basic protein and myelin basic protein isoform 4 are the only 2 proteins that are related to the same category of cellular component morphogenesis. The remaining 8 proteins are not directly related to each other.

Protein name	NCBInr accession no.	Method of identification #	MASCOT score *	MW, kDa	pI	Sequence Coverage, % †	Peptides assigned ‡	Change in expression Fold change ^	% §
<u>Translation, GO: 0006412</u>									
40S Ribosomal protein S18 [Rattus norvegicus]	gil47087103	LC ESI	148	17.7	11.5	26.3	4	1.30 ± 0.07	$\uparrow 30 \pm 7$
<u>Cellular component morphogenesis, GO: 0032989</u>									
Myelin-associated oligodendrocyte basic protein [Rattus norvegicus]	gil6981216	LC MALDI	86.7	19.3	12.3	26	3	3.70 ± 0.37	$\uparrow 270 \pm 37$
Myelin basic protein isoform 4 [Rattus norvegicus]	gil70166270	LC ESI	93.2	17.2	11.3	36.7	4	1.65 ± 0.28	$\uparrow 65 \pm 28$
<u>Glycolysis, GO:0006096</u>									
Fructose-bisphosphate aldolase C [Rattus norvegicus]	gil6978489	LC ESI	582	39.7	7	35.8	17	1.53 ± 0.35	$\uparrow 53 \pm 35$

	<u>Cell surface receptor linked signal transduction, GO: 0007166</u>								
Ankyrin binding cell adhesion molecule neurofascin [Rattus norvegicus]	gil1842427	LC MALDI	132	132	6.2	9.8	4	1.65 ± 0.16	$\uparrow 65 \pm 16$
	<u>Intercellular signaling cascade, GO: 0007242</u>								
ras-Related C3 botulinum toxin substrate 1, isoform CRA_b [Rattus norvegicus]	gil149034945	LC MALDI	116	21.8	9.7	15.6	4	1.94 ± 0.02	$\uparrow 94 \pm 2$
	<u>Exocytosis, GO: 0006887</u>								
Vesicle associated membrane protein 2B [Rattus norvegicus]	gil4894188	LC ESI	182.8	14.6	5.3	23	5	3.26 ± 0.55	$\uparrow 226 \pm 55$
	LC MALDI								
	<u>Cation transport, GO: 0006812</u>								
Na+/K+ -ATPase alpha 2 subunit precursor [Rattus norvegicus]	gil6978545	LC MALDI	107.7	113.5	5.3	6.3	3	1.75 ± 0.15	$\uparrow 75 \pm 15$
	<u>Nucleobase, nucleoside, nucleotide and nucleic acid metabolic process, GO: 0006139</u>								
Dihydropyrimidinase-related protein 2 [Rattus norvegicus]	gil157786744	LC MALDI	134.6	62.6	6	10.7	3	4.03 ± 2.27	$\uparrow 303 \pm 227$

<u>Mitosis, GO: 0007067</u>							
Centromere protein F [Rattus norvegicus]	gil281371331	LC ESI	56.7	306.2	5	3.1	2 2.32 ± 0.66 ↑ 132 ± 66

#Method of identification: LC ESI: identified by nano C₁₈ liquid chromatography coupled with electrospray ionization ion-trap tandem mass spectrometry. LC MALDI: identified by nano C₁₈ liquid chromatography coupled with matrix assisted laser desorption ionization time-of-flight/time-of-flight mass spectrometry.

*MASCOT score: Sum of the ion scores of the peptides assigned to the identified proteins.

†Sequence Coverage %: Sum of the percentages of amino acid sequences covered by the peptides assigned to the identified proteins.

‡Peptide assigned: number of peptides assigned to the identified protein with ion scores above the 95% cutoff values (p < 0.05).

^ Fold change: It is defined as the mean fold changes of the expression levels of the differentially expressed proteins plus/minus standard deviation. Fold change was calculated as = peak area of the peptide assigned from MeHg group / peak area of the peptide assigned from vehicle group. Six biological replicates were used.

§Percentage change in expression, % expressed as the mean of percentage change of the peak area of peptides assigned to the protein identified, \pm standard deviation. \uparrow : increased in percentage. \downarrow : decreased in percentage. The percentage change in expression for each peptide assigned = (peak area of the peptide assigned from MeHg group – peak area of the peptide assigned from vehicle group) / peak area of the peptide assigned from vehicle group $\times 100\%$. 6 biological replicates were used.