

Supplementary information

Hippocampal metabolism of amino acids by L-amino acid oxidase is involved in fear learning and memory

Kento Usuda, Takahiro Kawase, Yuko Shigeno, Susumu Fukuzawa, Kazuki Fujii, Haolin Zhang, Takamitsu Tsukahara, Shozo Tomonaga, Gen Watanabe, Wanzhu Jin & Kentaro Nagaoka

Content

Figure S1. Hot plate test.

Figure S2. Original gel image in Fig. 5

Table S1. The result of measurement of 19 L-amino acids in plasma.

Table S2. The list of metabolites that showed significant difference between WT and LAO KO hippocampus.

Table S3. Multiple reaction monitoring transitions and individual parameters for the neurotransmitters analysis by LC-MS/MS.

Table S4. The list of primers.

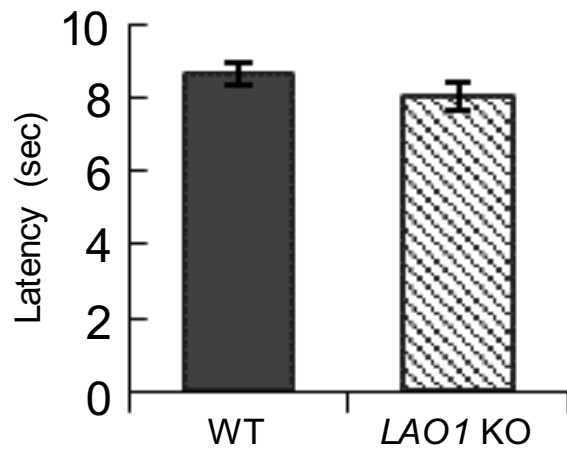


Figure S1. Hot plate test. Mice (male WT mice: n=8, male LAO1 KO mice: n=8) were placed on a hot plate preheated to 55° C until the mice manifested a nociceptive behaviour

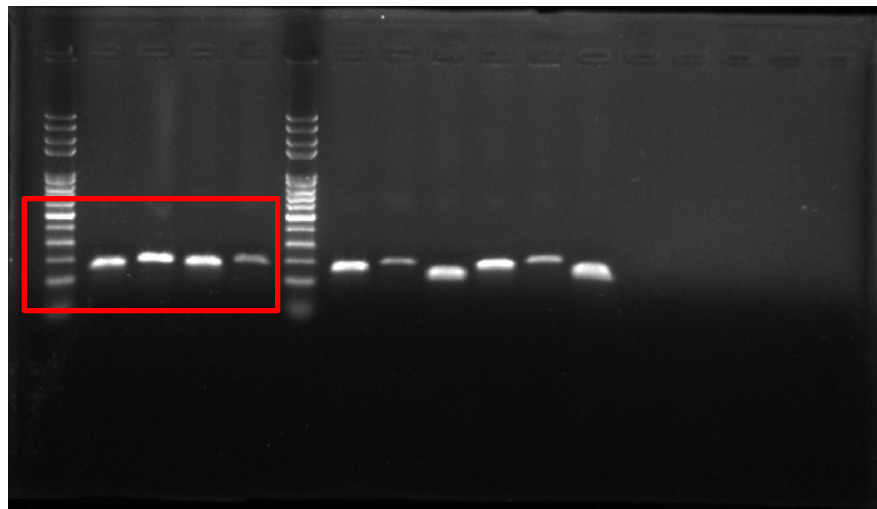
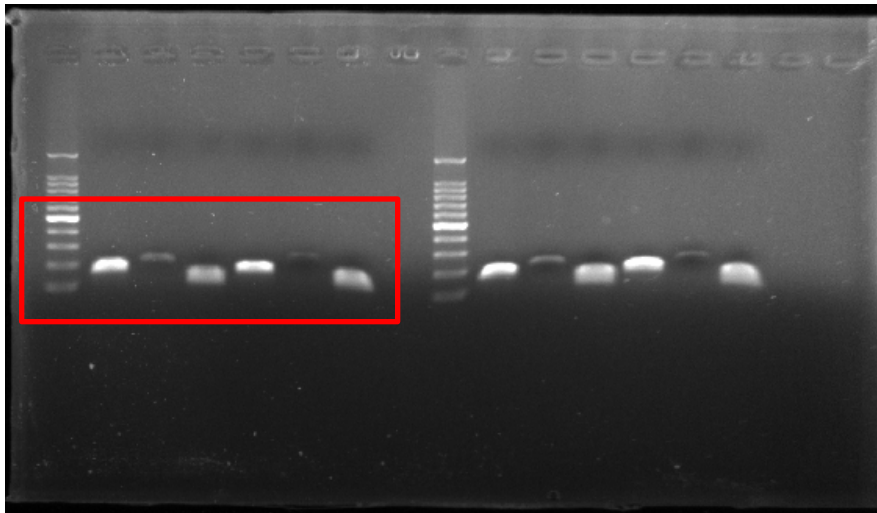


Figure S2. Original gel image in Fig. 5.

	WT	LAO KO	p value
Alanine	39.23 ± 3.03	50.21 ± 2.34	p < 0.05
Glycine	41.05 ± 2.41	48.93 ± 2.84	N.S
Valine	31.04 ± 2.28	39.58 ± 2.75	p < 0.05
Leucine	21.79 ± 1.91	28.27 ± 2.19	N.S
Isoleucine	12.17 ± 1.02	14.68 ± 1.10	N.S
Threonine	20.59 ± 2.35	26.39 ± 2.27	N.S
Serine	14.15 ± 1.27	19.03 ± 2.00	N.S
Proline	16.56 ± 1.21	22.33 ± 1.78	p < 0.05
Asparagine	5.76 ± 0.80	10.10 ± 1.48	p < 0.05
Aspartic acid	2.89 ± 0.28	4.01 ± 0.44	N.S
Methionine	8.76 ± 0.60	11.08 ± 0.86	N.S
Glutamic acid	10.04 ± 0.80	11.88 ± 0.88	N.S
Phenylalanine	9.15 ± 0.74	14.45 ± 1.75	p < 0.05
Glutamine	39.53 ± 3.34	57.57 ± 8.00	N.S
Ornithine	11.44 ± 0.90	16.66 ± 1.88	p < 0.05
Lysine	28.19 ± 2.84	34.97 ± 2.82	N.S
Histidine	8.36 ± 0.12	9.55 ± 0.65	N.S
Tyrosine	9.61 ± 0.78	13.23 ± 2.23	N.S
Tryptophan	4.97 ± 0.31	4.91 ± 0.52	N.S
LNAA	105.84 ± 7.13	135.75 ± 8.75	p < 0.05
Phe/LNAA	0.086 ± 0.016	0.106 ± 0.008	N.S
Tyr/LNAA	0.091 ± 0.006	0.095 ± 0.012	N.S
Trp/LNAA	0.047 ± 0.002	0.036 ± 0.004	p < 0.05

Table S1. The result of measurement of 19 L-amino acids in plasma.

Data are presented as means (nmol / L) ± S.E.M. p < 0.05, unpaired t-test.

N.S: no significant difference.

	WT	LAO KO
Mannitol	1 ± 0.18	1.73 ± 0.19
Arabitol	1 ± 0.16	1.62 ± 0.20
Malic acid	1 ± 0.13	1.99 ± 0.26
Isocitric acid&Citric acid	1 ± 0.15	2.01 ± 0.29
5-Hydroxyindoleacetic acid	1 ± 0.11	2.04 ± 0.14
Erythritol	1 ± 0.15	1.95 ± 0.13
Uracil	1 ± 0.18	2.12 ± 0.21
Niacinamide	1 ± 0.16	1.74 ± 0.11
Lyxose	1 ± 0.17	1.78 ± 0.17
Fumaric acid	1 ± 0.17	1.71 ± 0.23
(+/-)-erythro-Isoleucine	1 ± 0.16	2.03 ± 0.20
N-Acetylglutamic acid	1 ± 0.13	1.92 ± 0.22
Beta-Alanine	1 ± 0.23	2.01 ± 0.24
O-Phosphoethanolamine	1 ± 0.17	2.14 ± 0.31
Adenine	1 ± 0.14	1.94 ± 0.22
Glycine	1 ± 0.19	2.04 ± 0.28
Indoxyl sulfate	1 ± 0.32	2.30 ± 0.28
Galactitol	1 ± 0.15	2.13 ± 0.44
Dopamine	1 ± 0.18	2.13 ± 0.28
Hypotaurine	1 ± 0.16	2.00 ± 0.26
Acetoacetic acid	1 ± 0.41	0.02 ± 0.005
Alpha-ketoisovaleric acid	1 ± 0.41	0.004 ± 0.0007
Phenylpyruvic acid	1 ± 0.09	0.05 ± 0.013
Sarcosine	1 ± 0.16	2.00 ± 0.26
2-Aminoisobutyric acid	1 ± 0.16	2.00 ± 0.26
Phenylalanine	1 ± 0.17	3.12 ± 0.64
Cadaverine	1 ± 0.31	3.91 ± 1.18
3-Hydroxybutyric acid	1 ± 0.17	2.62 ± 0.59
5-Sulfosalicylic acid	1 ± 0.16	2.35 ± 0.53
threo-3-Hydroxy-aspartic acid	1 ± 0.16	2.35 ± 0.53
N-Formyl-L-methionine	1 ± 0.16	2.35 ± 0.53
2-Methyl-1-propylamine	1 ± 0.16	2.55 ± 0.48
Deoxyguanosine	1 ± 0.16	2.55 ± 0.48
Pelargonic acid	1 ± 0.27	2.49 ± 0.42
Homoserine	1 ± 0.17	1.76 ± 0.21
Serine	1 ± 0.17	1.62 ± 0.20
Methionine sulfone	1 ± 0.99	6.57 ± 1.76
N-Acetyl-b-D-galactosamine	1 ± 0.36	2.18 ± 0.20
Glutamic acid	1 ± 0.29	2.02 ± 0.14
Allantoin	1 ± 0.37	2.02 ± 0.19
Tyrosine	1 ± 0.16	2.49 ± 0.55

Table S2. The list of metabolites that showed significant difference between WT and LAO KO hippocampus.

Analytes	m / z	Cone voltage (V)	Collision Energy (V)
Tyrosine ¹³ C ₉ , ¹⁵ N ₁	192.3 > 145.2	25	18
Acetylcholine	146.0 > 87.2	25	18
Serotonin	177.1 > 160.2	18	18
Dopamine	154.0 > 137.2	24	18

Table S3. Multiple reaction monitoring transitions and individual parameters for the neurotransmitters analysis by LC-MS/MS.

Primer	Sequence 5' to 3'
PK Forward	ggtgtttgcacatcttca tcc
PK Reverse	ccttctctgcagga atctca
Pah Forward	ccaccaggettta aag atcc
Pah Reverse	cagagtcctgaaca ccg ttcc
Bpgm Forward	taatttatcgtggc cca gacg
Bpgm Reverse	acgggaggagcaga aaa gt tc
Ddc Forward	caggcttacatccg aaa gc ac
Ddc Reverse	agcagaccaacca aga at ga
Chrm1 Forward	accagcatcaggac cgg aaa
Chrm1 Reverse	tttgccgatccct ctgaca
AchE Forward	cactcctcttctcctcctc
AchE Reverse	gtggtagcatccaacac tc c
LAO1 Forward	tggccaagaagagt gga atc
LAO1 Reverse	agctcccactaccacca ca c
β -Tublin Forward	gctgaccagtgcac gc
β -Tublin Reverse	aaacctggggggct ggg t
Dsp Forward	gctgaagaacactc tagccca
Dsp Reverse	actgctgtttcctc tga gaca

Table S4. The list of primers.

PK: Pyruvate kinase, Pah: Phenylalanine hydroxylase, Bpgm: 2,3-bisphosphoglycrate mutase, Ddc: Aromatic L-amino acid decarboxylase, Chrm1: muscarinic M1 Ach receptor, AchE: Acetylcholine esterase, Dsp:desmoplakin