

Comprehensive Analysis of Neurotransmitters from Regenerating Planarian Extract Using UHPLC-MS/SRM Method

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Abstract

Absolute quantification of neurotransmitters (NTs) from biological systems is imperative to track how changes in concentration of active neurochemicals may affect biological behavior. A stable isotope dilution ultrahigh performance liquid chromatography/mass spectrometry/selected reaction monitoring (UHPLC-MS/SRM) assay has been developed for a sensitive and quantitative assessment of NTs in planaria. We used this method for the simultaneous quantification of sixteen NTs from both sexual, asexual and regenerating planarians. Each of the sixteen well resolved analytes showed a linear relationship between concentrations (0.78–50 ng/mL), regression coefficients higher than 0.97, accuracy (91–109%) and low coefficients of variation (CVs). The inter-day CVs for the lowest quality controls (1.56 ng/mL) were in the range between 2–11%. The levels of most of the NTs were similar in both sexual and asexual planarians except for glutamic acid, which was about two-fold higher in asexual compared to sexual planarian. Further, the levels of melatonin and epinephrine were lower than the limit of quantification (LOQ) in both the strains. We also identified high levels of serotonin and failed to detect tryptamine suggesting that the pathway essential for the conversion of tryptophan to tryptamine is absent in planarians. Interestingly, we also found high levels of dopamine and L-DOPA in regenerating planarians suggesting their possible role in regeneration. For the first time, we developed novel methodology based on UHPLC-MS/SRM and quantified sixteen NTs with high sensitivity and specificity from sexual and asexual strain of planarian *Schmidtea mediterranea*. This method will also have great application in quantifying various neurotransmitters with great precision in different model systems.

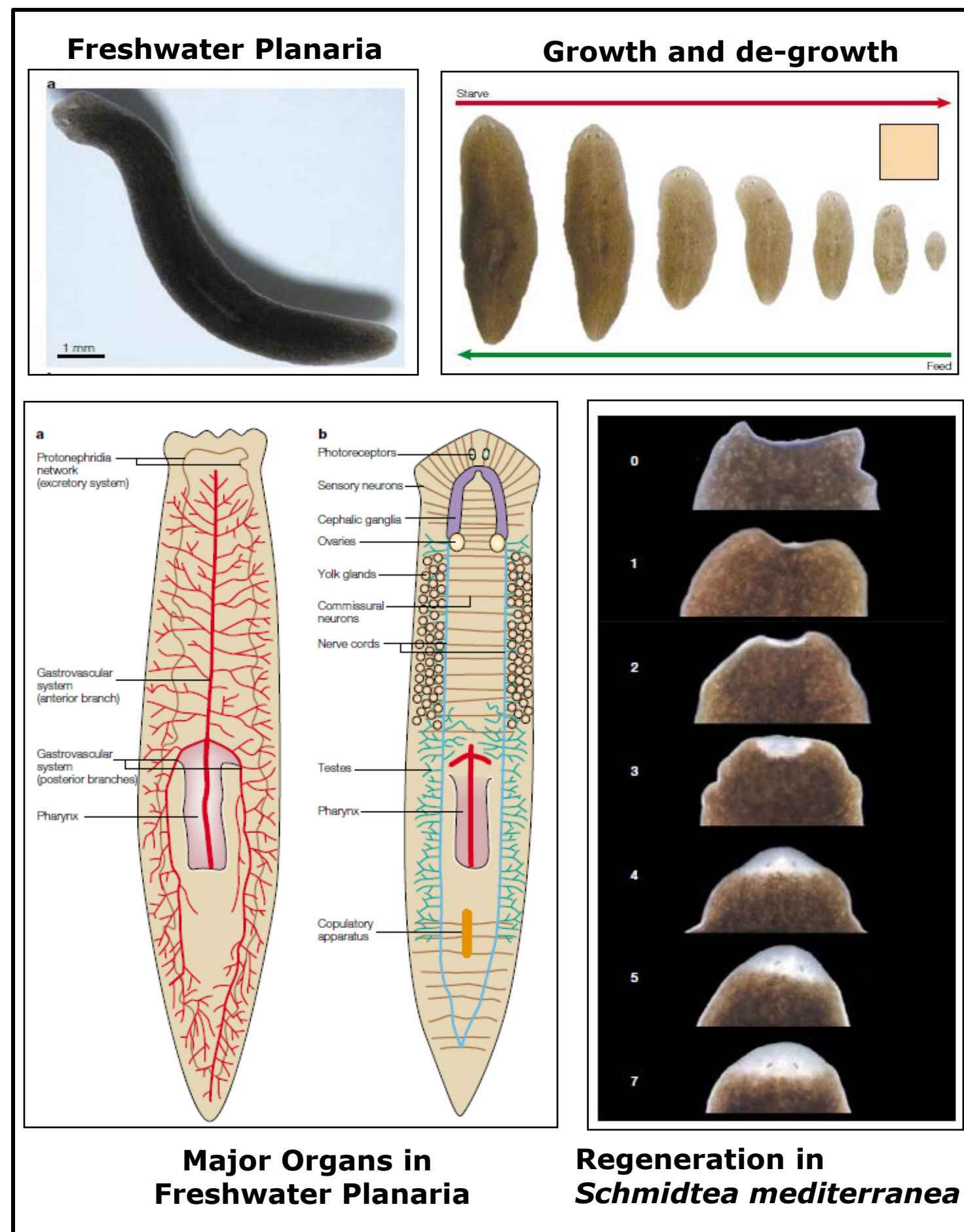
Introduction

Capable of regenerating the lost tissue or organ. Regenerative capacity is such that 1/273rd piece of the animal is capable of regenerating the whole animal

Planarians undergo both sexual and asexual reproduction

Neoblasts (stem cells) that is maintained in the planarian throughout adult life, these cells are the only proliferating cells in planaria

Good model to study stem cell biology



Nature Reviews 3, 210-219, 2002.

Role of Neurotransmitters

Chemical NTs and hormones occupy key positions in the regulation of physiological processes

A diverse array of NTs and neuromodulators control and affect brain function

Several groups of NTs like amino acids, catecholamines and neuropeptides are present in the biological system

Communications of cells in the neural networks completely rely on the maintenance of balance among the concentrations of such neurochemicals

Disturbed balance between two or more such NTs in the brain has already been observed in many diseases like Parkinson's disease, Alzheimer's disease and Schizophrenia

Neurotransmitters in Planaria

Serotonin, dopamine, tyramine, histamine, norepinephrine and octapamine were reported in the flatworms

Major role in the muscle contraction and the spike activity in the CNS

Serotonin and dopamine are known to increase the regeneration in planaria

It has been shown that various enzymes like typtophan hydroxylase, tyramine β -hydroxylase in planarian *Dugesia japonica*

Nothing much is known about the species *Schmidtea mediterranea*

Sample preparation

• Three worms of each sexual and asexual were taken in eppendorf tube (in triplicates) and washed with water thrice.

• Worms were weighed without water in the accurate analytical balance.

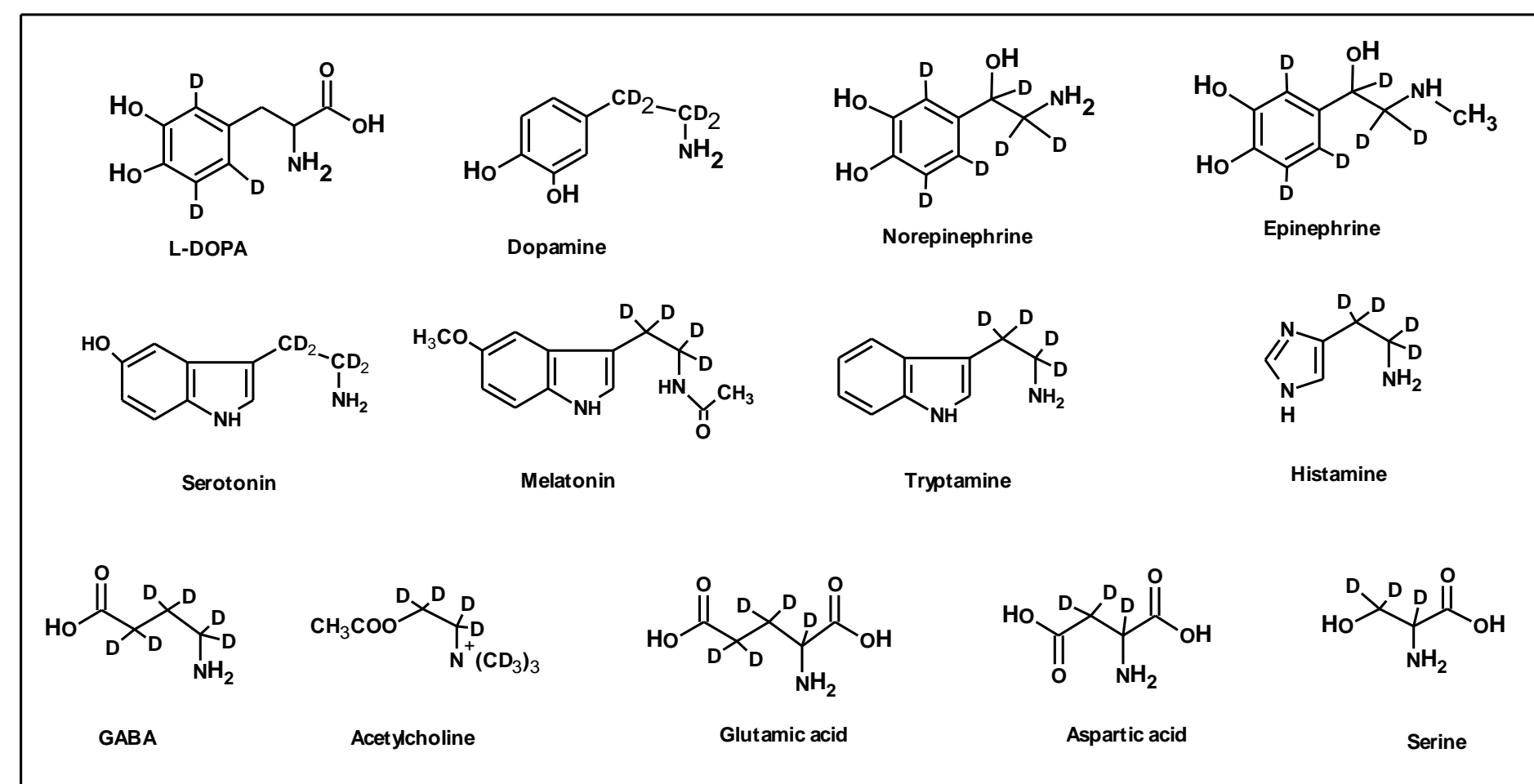
• Worms were crushed using plastic pestle in 200 μ l of acetone (0.1 % FA)

• Then Centrifuged (14000 rpm, 5 min) and transferred the supernatant to the fresh eppendorf and ISTDs (10 ng) were spiked.

• It was then dried in speed vacuum and reconstitute it in 50 μ l of 0.5% acetonitrile (0.1% FA) and transferred into the HPLC vial for the injection.

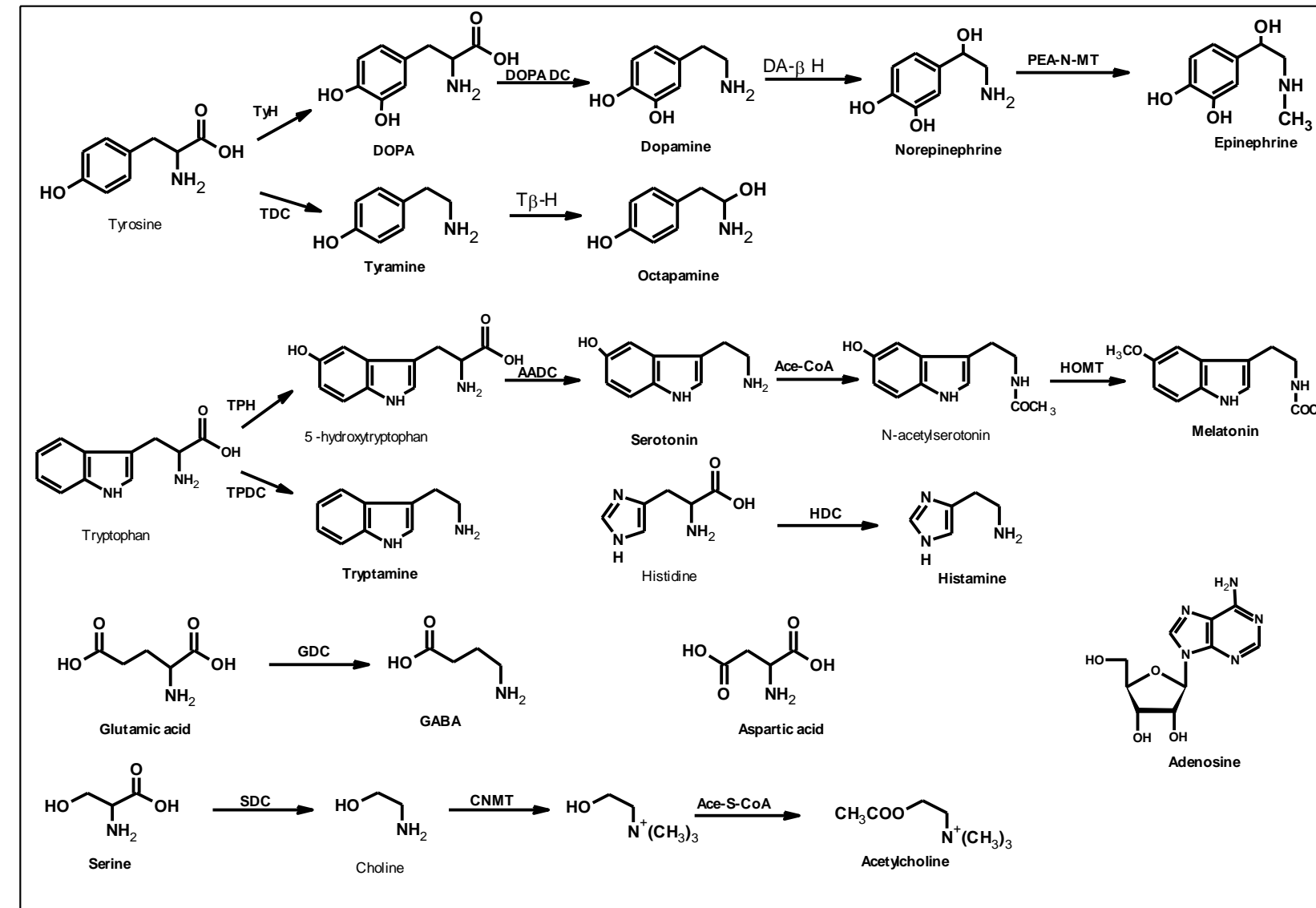
• 10 μ l was injected for the UHPLC-MS/SRM analysis.

ISTDs Used



Quantification of Neurotransmitters from Planarian extract

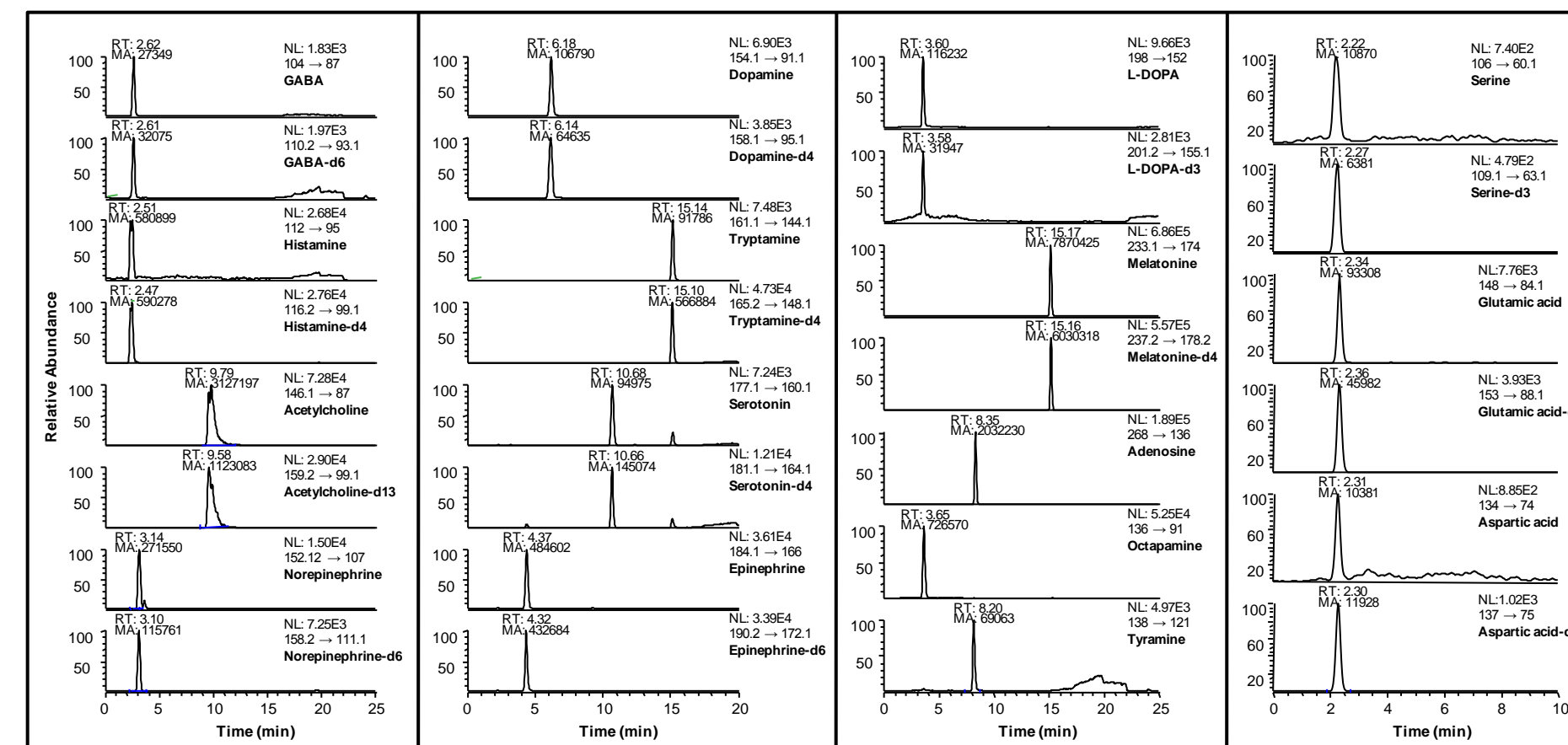
Biosynthetic pathways for the formation of NTs



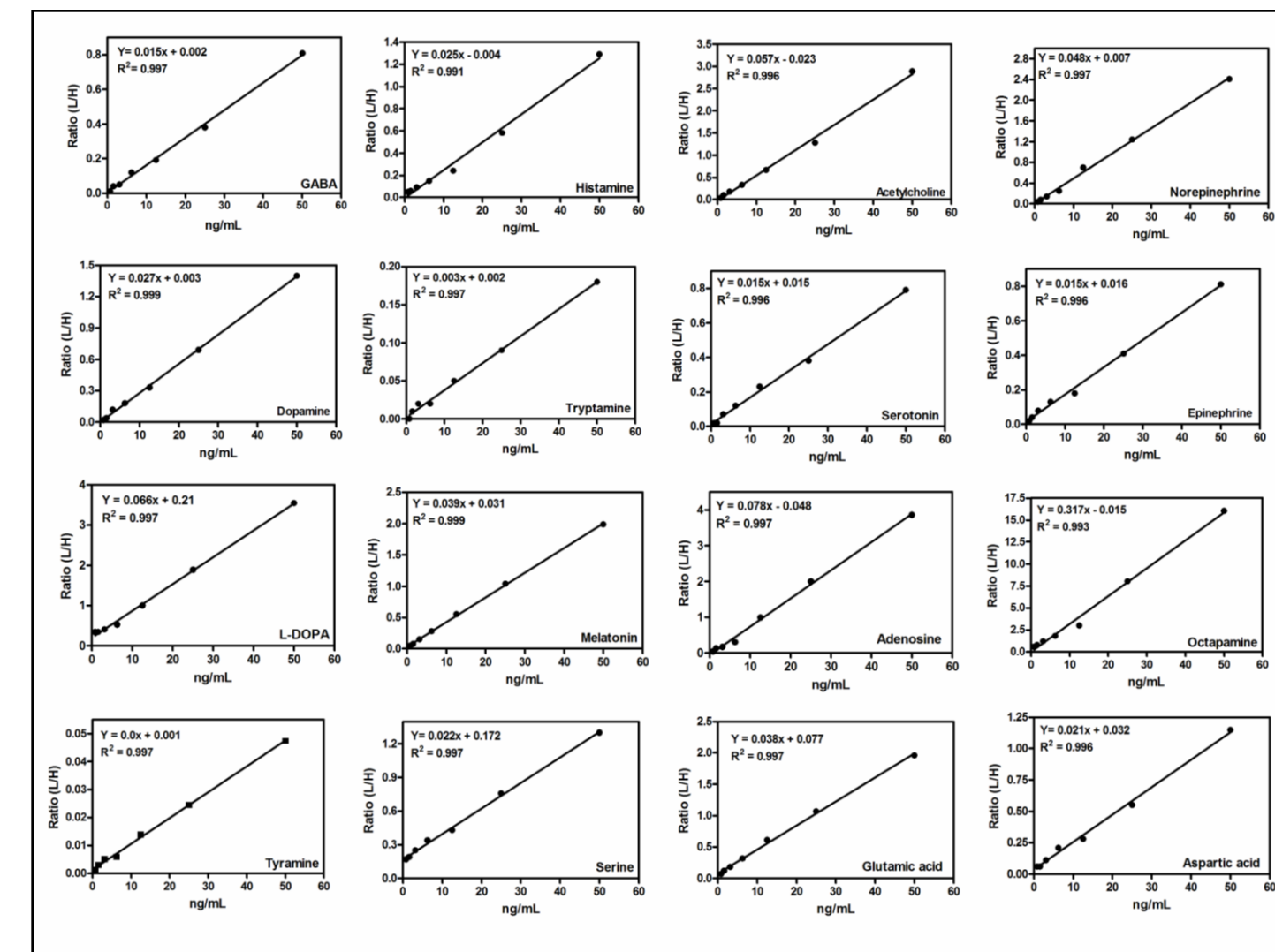
SRM Table for NTs analyzed

	Neurotransmitter	Parent ion (m/z)	Product ion (m/z)	Collision Energy (eV)	S-lens voltage	Retention Time (min)
1	GABA	104.08	87.05	7	38	2.62
2	GABA-d6	110.2	93.11	7	38	2.62
3	Histamine	112.08	95.07	14	43	2.51
4	Histamine-d4	116.2	99.137	14	43	2.51
5	Acetylcholine	146.113	87.07	13	46	9.79
6	Acetylcholine-d13	159.25	91.117	13	46	9.79
7	Norepinephrine	170.2 (152.116)	107.06	17	56	3.14
8	Norepinephrine-d6	176.2 (158.2)	111.115	17	56	3.14
9	Dopamine	154.119	91.07	23	40	6.18
10	Dopamine-d4	158.14	95.1	23	40	6.18
11	Serotonin	177.1	160.104	11	40	10.68
12	Serotonin-d4	181.14	164.131	11	40	10.68
13	Epinephrine	184.11	166.09	7	42	4.37
14	Epinephrine-d6	190.25	172.155	7	42	4.37
15	DOPA	198.08	152.092	13	51	3.6
16	DOPA-d3	201.2	155.116	13	51	3.6
17	Melatonin	233.106	174.09	14	57	15.17
18	Melatonin-d4	237.2	178.137	14	57	15.17
19	Tryptamine	161.1	144.105	12	36	15.14
20	Tryptamine-d4	165.2	148.131	12	36	15.14
21	Adenosine	268.08	136.062	18	64	8.35
22	Tyramine	138.08	121.077	10	44	8.2
23	Octapamine	154.18 (136.07)	91.073	17	64	3.65
24	Serine	106.08	60.1	10	40	2.22
25	Serine-d3	109.1	63.1	10	40	2.22
26	Glutamic acid	148.08	84.1	14	47	2.34
27	Glutamic acid-d5	153.09	88.1	14	47	2.34
28	Aspartic acid	134.06	74	12	43	2.31
29	Aspartic acid-d3	137.06	75	12	43	2.31

UHPLC-MS/SRM chromatogram of all sixteen NTs and thirteen ISTDs in HQC level



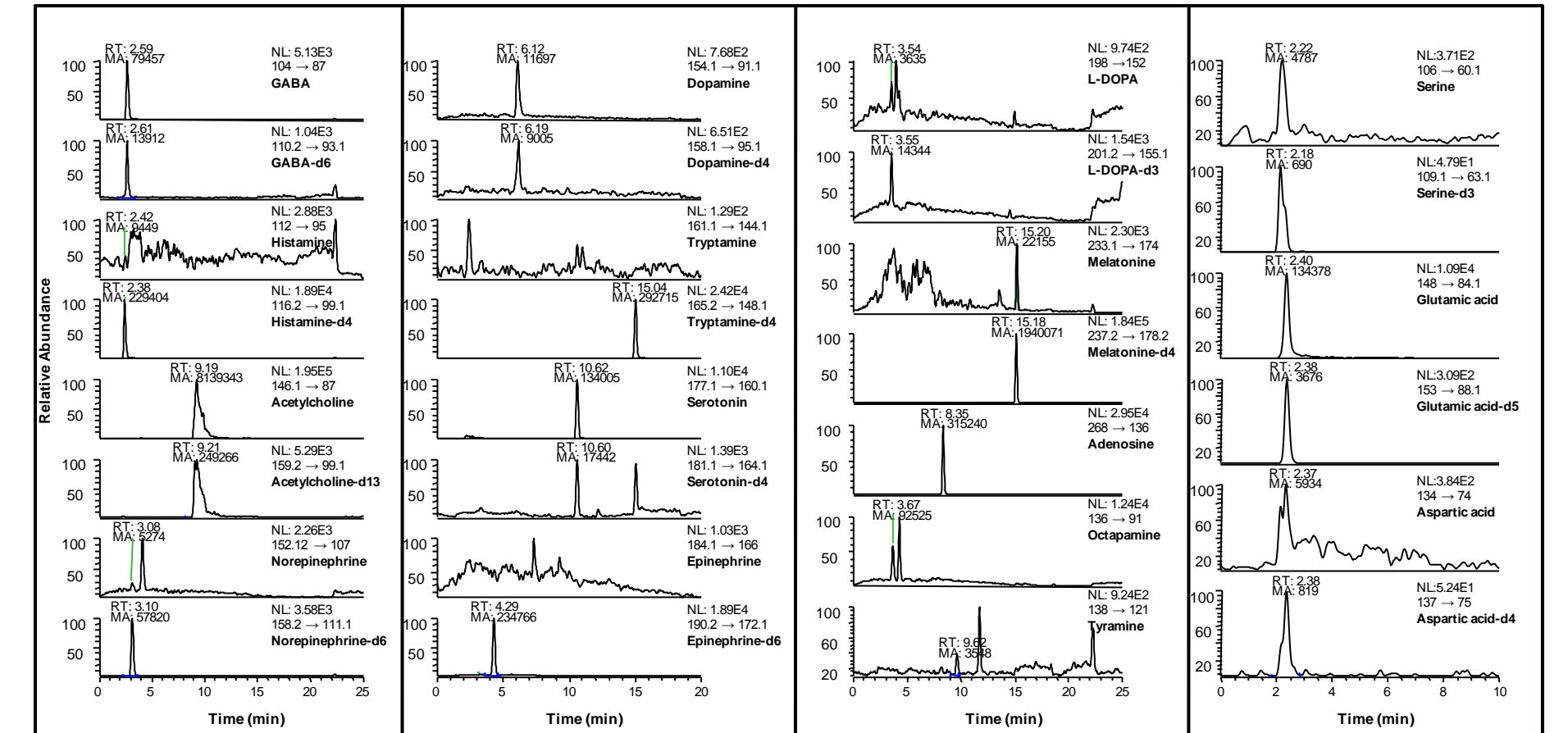
Standard curves and regression line analysis of sixteen NTs



Validation for UHPLC-MS/SRM analysis of NTs on five different days

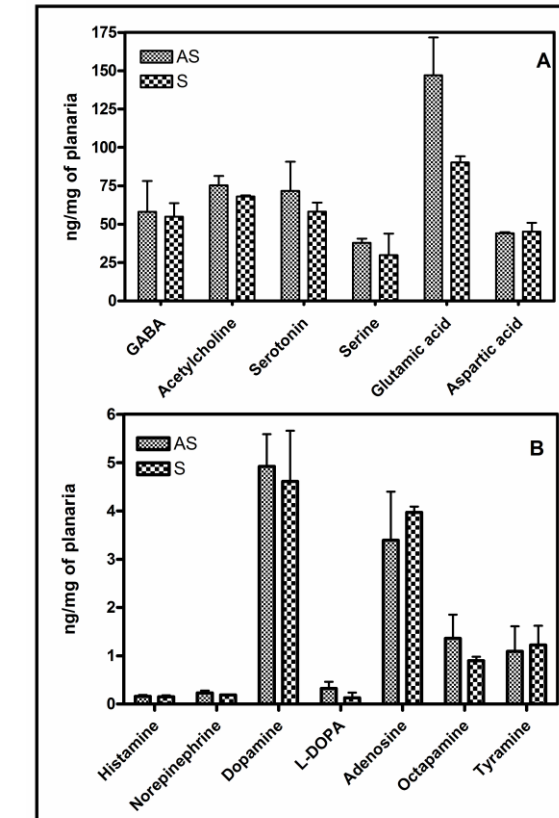
	LOQ	GC	MC	HQC
NTs	1.56	1.52	20.00	40.00
NTs (d5)	1.56	1.52	20.00	40.00
NTs (d5)	1.56	1.52	20.00	40.00
NTs (d5)	1.56	1.52	20.00	40.00
NTs (d5)	1.56	1.52	20.00	40.00

UHPLC-MS/SRM chromatogram of all sixteen NTs from asexual planarian

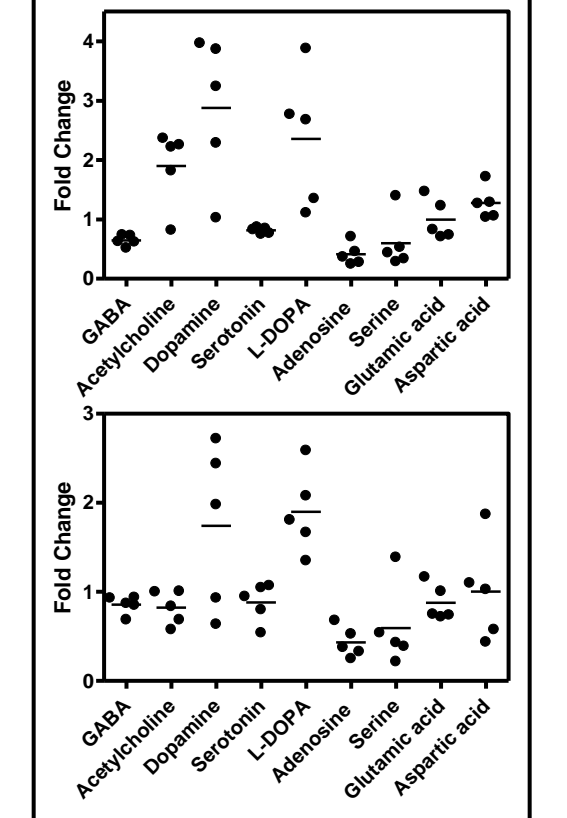


Quantification of neurotransmitters from planaria

Sexual and asexual



Posterior & Anterior regeneration



Conclusions

Stable isotope dilution UHPLC-MS/SRM assay was developed and validated to evaluate the concentrations of sixteen NTs from the planarian extract

The LOQ for each analyte was 1.56 ng/mL and standard curves were linear in the range of 0.78-50 ng/mL.

The assay was sensitive, specific and very robust

Various NTs were up regulated in the regenerating planarians suggesting their role in regeneration

Acknowledgments

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