Running Title: Neurotransmitter metabolism in Alzheimer's brain

Comparing neurotransmitter metabolism in the brain of patients with symptomatic and asymptomatic Alzheimer's disease and healthy individuals.

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ABSTRACT

Three of the four treatments for Alzheimer's disease (Donepezil, Rivastigmine and Galantamine) are cholinesterase inhibitors that target the pathological reduction of acetylcholine levels, while the fourth treatment Memantine is an NMDA antagonist. In this study, six neurotransmitters and nine pathway-associated metabolites were measured in 43 human brain samples from the autopsy cohort of the Baltimore Longitudinal Study of Aging (BLSA). Tissue samples were obtained from three groups of BLSA participants, AD (n=14), controls (n=14) and asymptomatic AD (ASYMAD; n=15) i.e. individuals with significant AD neuropathology at death but with no evidence of cognitive impairment during life. Three brain areas were studied, middle frontal gyrus (MFG), inferior temporal gyrus (ITG) and the cerebellum. When analysing the levels of neurotransmitters and associated metabolites in the brain, 6 of 15 were shown to be significantly associated with disease ($p<0.005$). Dopamine decreased in the ASYMAD group suggesting a decrease of this neurotransmitter before cognition is impaired (fold change; FC=0.78, $p=4.1\times10^{-3}$). In the AD brain, changes were mainly seen in the ITG with increases observed in GABA, arginine and ornithine and decreases in aminobutanal, aspartate, glutamate, guanidinobutanoate, glycine and guanosine. Taken together, these results indicate that AD pathology is associated with perturbations in several neurotransmitter systems. These findings may have important implications for drug repurposing and complementary treatment of symptoms.

INTRODUCTION

Dementia is a devastating illness for both patients and their families, with Alzheimer's disease (AD) estimated to account for up to 80% of total dementia cases. The 'World Alzheimer's report 2015' estimates that there are approximately 46 million AD patients worldwide, this number is expected to rise to over 130 million by the middle of the century (1). As well as a significant human cost, AD also represents a major financial burden with worldwide costs related to AD expected to reach $1 trillion dollars in 2018 (1).

Cholinesterase inhibitors make up three of the four approved AD treatments (Donepezil, Rivastigmine and Galantamine) making inhibition of acetylcholinesterase the leading therapeutic strategy for the treatment of AD symptoms (2, 3). There is a significant body of literature suggesting that the cognitive deficits associated with Alzheimer's disease are the result of lower levels of acetylcholine in the brain resulting from dysfunction of cholinergic neurons (4-6). The role of non-cholinergic neurotransmitter systems in AD pathogenesis has received less attention. While levels of non-cholinergic neurotransmitters in the brain have been associated with Alzheimer's pathology (7-11), their role in mediating the onset of symptoms is less well understood. In this study, we analysed data from non-targeted metabolomics to compare differences in neurotransmitters and neurotransmitter-associated metabolite levels in brain tissue samples from the autopsy cohort of the Baltimore Longitudinal Study of Aging (BLSA). We studied three groups of BLSA participants, AD, cognitively normal controls and 'asymptomatic AD' (ASYMAD; i.e. individuals with significant AD neuropathology at death but with no evidence of cognitive impairment during life). We studied three distinct brain regions in these individuals that are differentially effected by core pathological features of AD, the inferior temporal gyrus that is especially vulnerable to neurofibrillary tau tangles, the middle frontal gyrus which is susceptible to the accumulation amyloid plaques and the cerebellum which is resistant to classical AD pathology (12). Our main aim was to test associations between AD neuropathology and metabolism in a variety of neurotransmitter systems.

METHODS

Sample Information

The BLSA is a prospective, ongoing cohort study of community-dwelling volunteer participants in Baltimore begun in 1958. As such, it is among the largest and longest-running longitudinal studies of aging in the United States (13, 14). In general, at the time of entry into the study, participants had no physical or cognitive impairment. Detailed examinations, including neuropsychological assessments and neurological, laboratory, and radiological evaluations, were conducted every 2 years. Since 2003, participants older than 80 years have received yearly assessments. Written informed consent was obtained at each visit, and the study was approved by the local Institutional Review Board and the National Institute on Aging. After each visit, cognitive status was considered at consensus diagnosis conferences relying on information from neuropsychological tests as well as clinical data as described previously (15). Diagnoses of dementia and Alzheimer's disease (AD) were based on DSM-III-R (16) and the NINCDS-ADRDA criteria (17) respectively.

Brain tissue samples were collected through the autopsy sample of the BLSA. The autopsy program of the BLSA was initiated in 1986. We have previously described the study protocol in detail. Briefly, the mean age at death in the autopsy sample is 88.3 ± 7.3 years (range 69.3–103.2), and the mean interval between last evaluation and death is 8.7± 6.7 months (18). As reported previously, the autopsy subsample is not significantly different from the BLSA cohort as a whole in terms of the rates of dementia and clinical stroke (19). Table 1. describes the demographic characteristics of the participants whose brain tissue samples were used in this study.

Data acquisition

The majority of the data described in this paper was generated previously, the study with a detailed description of the acquisition, analysis and annotation of thirteen metabolites: Tyrosine, L-DOPA, Dopamine, Aminobutanal, Arginine, Aspartate, GABA, Glutamate, Glutamine, Guanidinobutanoate, Glycine, Guanosine and Ornithine (20). Additional metabolite levels were measured (serotonin and tryptophan) for this cohort using the Biocrates P180 platform. To extract metabolites from brain tissue, samples were homogenized using Precellys® with ethanol phosphate buffer. Samples were centrifuged and the supernatant was used for analysis. The fully automated assay was

based on liquid chromatography-tandem mass spectrometry (LC-MS/MS; amino acids) using a SCIEX 4000 QTrap® mass spectrometer (SCIEX, Darmstadt, Germany) with electrospray ionization. Brain tissue concentration was absolute concentration expressed as nmol/mg tissue

Statistical methods and pathway mapping

To compare the fifteen metabolites among 3 groups (CN, ASYMAD and DEMENT), we used Mann–Whitney U test for pairwise comparisons. To control for type 1 errors, p-values were corrected with Benjamini-Hochberg performed in 'R' and this were reported with a start in table 2. In the results section we reported all metabolites with $p<0.05$. The relationship of metabolite abundance to measures of neuritic plaque and neurofibrillary tangle burden in the brain (as described by CERAD and Braak scores respectively) were determined by calculating the Pearson's product-moment correlation coefficient.

Pathway mapping was performed in Cytoscape v3.4.0, the architecture was determined by metabolic interactions defined in the Kyoto Encyclopaedia of Genes and Genomes (KEGG). Within the network, node size is directly proportional to the fold change in metabolite abundance, with edge thickness directly proportional to the partial correlation of the two nodes it is connecting.

RESULTS

Region specific analysis in the ASYMAD versus control group

Fifteen metabolites consisting of six neurotransmitters and nine associated metabolites were compared in the ASYMAD group versus the control group. In detail (Table 2): Tyrosine, L-DOPA, Dopamine, Tryptophan, Serotonin, Aminobutanal, Arginine, Aspartate, GABA, Glutamate, Glutamine, Guanidinobutanoate, Glycine, Guanosine and Ornithine were measured. In the MFG, dopamine was decreased (FC=0.78, $p=4.1\times10^{-3}$) and in the ITG aminobutanal and guanosine were also decreased (FC=0.86, $p=2.2\times10^{-2}$ and FC=0.72, $p=1.7\times10^{-2}$ respectively) (Table 2, Sup Figure 1).

Region specific analysis in the AD versus control group

In the comparison of AD versus control, changes were observed mainly in the ITG. In the ITG excitatory neurotransmitters serotonin, glutamate and aspartate exhibited a lower abundance ($p<0.05$ & FC=0.8) in AD patients. Also in the ITG, inhibitory neurotransmitters glycine and GABA were decreased and increased respectively ($p<0.05$, FC=0.8 and 1.2). A number of neurotransmitter precursors were increased: ornithine, arginine and tryptophan (all $p<0.05$) with arginine also significant at $p<0.005$, whilst guanidobutanoate, guanosine, aminobutanal were all significantly decreased (all $p<0.005$) in the ITG of AD patients (Table 2, Figure 1).

Figure 1 Showing pathway analysis of the association of neurotransmitter metabolism to Alzheimer's disease in human brain. Metabolites significantly increased in abundance ($p<0.05$) and shown as green triangles and metabolites significantly decreased in abundance ($p<0.05$) and shown as red chevrons with the size representing the magnitude of the change. Grey circles represent metabolites that were not significantly associated with disease. **A)** shifts observed in the cerebellum, **B)** shifts observed in the inferior temporal gyrus, **C)** shifts observed in the middle frontal gyrus.

In the MFG dopamine precursors L-DOPA and tyrosine were the only metabolites to be increased with disease. An increase in L-DOPA was the only significant difference observed in the cerebellum (Table 2, Sup Figure 1).

Region specific analysis in the AD versus ASYMAD

In the comparison of AD versus ASYMAD, two changes were observed in the MFG: GABA was increased (FC=1.23, $p=3.7\times10^{-2}$) and guanidobutanoate was decreased (FC=0.78, $p=4.3\times10^{-3}$). In the ITG, guanidobutanoate was also decreased (FC=0.69, $p=1.2\times10^{-4}$) (Table 2, Sup Figure 1).

Correlation of metabolite abundance and measures of Alzheimer's pathology

When examining the relationship between metabolite abundance and measures of pathology and cognitive performance several weak but significant correlations we observed. These data were collected during life as explained in the methods section. Of the 15 metabolites measured, all (with the exception of dopamine, glutamate and glutamine) correlated with Braak and CERAD scores in all regions ($r^2> 0.2$, $p<0.05$) (Supplemental Table 1). Correlation analysis to investigate the relationship between metabolite abundance and cognitive performance showed that arginine, aspartate, aminobutanal and guanidobutanoate correlated with MMSE ($r^2> 0.2$, $p<0.05$) in all regions (Supplemental Table 2), GABA, aspartate, tyrosine, DOPA, ornithine, arginine, guanidobutanoate and aminobutanal

correlated with Benton's visual retention index ($r^2 > 0.2$, $p < 0.05$) (Supplemental Table 2) and guanidobutanoate correlated with the Boston naming score.

DISCUSSION

The metabolism of neurotransmitters is an important consideration in the pathology of neurological diseases. In this study we measured directly in human brain tissue four key excitatory neurotransmitters dopamine, glutamate, serotonin and aspartate as well as the abundance of three inhibitory neurotransmitters, serotonin, glycine and GABA (Table 2, Figure 1). We wanted to test if any observed modifications in neurotransmitter pathways were associated with the asymptomatic and AD groups, and if specific brain regions exhibited unique differences in neurotransmitter metabolism.

Dopaminergic depletion in brains with neuropathology and normal cognition.

Dopamine is a catecholamine neurotransmitter (21, 22) which plays several important roles in the brain acting via 4 distinct pathways, the mesolimbic, mesocortical, nigrostatial and tuberoinfundibular pathways. These pathways are responsible for regulating mood, and aiding cognitive and motor function with impairment of this system potentially causing depression (23), memory loss (24) and impaired motor control observed in patients with Alzheimer's disease.

Dopamine does not cross the blood brain barrier and is synthesised in two steps from the essential amino acid tyrosine, with the initial conversion of tyrosine to L-DOPA catalysed by tyrosine hydroxylase (TH) with the subsequent conversion of L-DOPA to dopamine catalysed by aromatic amino acid decarboxylase (AAAD). The greatest reduction in dopamine in the MFG was observed in the asymptomatic patients (Figure 2, Sup Figure 1). In the MFG this pathway shows an increase in the abundance of tyrosine and L-DOPA, the precursors of dopamine, followed by a reduction in the abundance of dopamine, suggesting a decrease in the abundance or activity of both TH and AAAD.

Figure 2 Potential modifications to synaptic transmission based on alterations in metabolism associated with Alzheimer's pathogenesis. (A) Effects of decreased dopamine synthesis on synaptic transmission. (B) Effects of decreased serotonin synthesis on synaptic transmission. (C) Effects of disrupted GABA metabolism on synaptic transmission. Metabolites or processes shown in **red** are decreased in abundance, **blue** are increased, **black** were measured but unchanged, and **grey** were non measured intermediates, solid lines represent metabolic reactions and dashed lines represent protein protein interactions. AAAD: aromatic amino acid decarboxylase, ABAD: aminobuteraldehyde dehydrogenase, AGase: arginase, alpha-syn: alpha-synuclein, GDCase: glutamate decarboxylase, ODase: ornithine decarboxylase, PAT: putrescine aminotransferase, THL: tyrosine hydroxylase, TM: tryptophan monooxygenase.

The activity of both TH and AAAD are inhibited by alpha-synuclein (25), a protein that has been shown to play important pathological roles in a range of neurodegenerative diseases including Parkinson's (26), Alzheimer's (27) and Lewy body dementia (26-28). Studies have also shown that soluble intraneuronal alpha-synuclein, in the absence of Lewy body pathology is increased in abundance by up to two-fold in the brains of AD patients (29). This suggests a plausible molecular mechanism by which alpha-synuclein may modulate brain dopamine concentrations in AD by inhibiting conversion of tyrosine to L-DOPA and of L-DOPA to dopamine. Reduced dopamine could reduce the amount of the neurotransmitter released into the synaptic cleft during synaptic transmission leading to impaired signal transduction (Figure 2). The data (Table 2) shows that shifts in dopamine metabolism are greater in the middle frontal gyrus in the ASYMAD group, suggesting that the changes in dopamine metabolism could occur before symptoms such as memory loss occur (30).

Neurotransmission inhibition in the inferior temporal gyrus in AD

The inhibitory neurotransmitter serotonin and its precursor tryptophan were measured in this study and decreased in the ITG of AD patients ($p<0.05$); but were not significant at $p<0.005$ (Table 2, Sup Figure 1M). Serotonin is synthesised from the essential amino acid tryptophan by tryptophan monooxygenase (TM) and aromatic amino acid decarboxylase (AAAD). As stated above, alpha-synuclein which is increased in the brains of AD patients (29) has been shown to inhibit the action of AAAD (25) suggesting a potential mechanism for the decreasing trend in serotonin synthesis that was observed in this study. The association of serotonin to the AD brain is interesting because recent reports have shown that antidepressants such as Trazodone, a serotonin antagonist, could maintain neural integrity and so this drug has been proposed to treat dementia (31, 32).

Glutamate was also reduced in the ITG of AD patients, this was surprising as glutamate activation of N-methyl-D-aspartate (NMDA) on the post synaptic neuron and its excite-toxicity have long been implicated in the pathology of AD (33-35). The glutaminergic system in the brain leads to impairment of a range of neurological functions, including fast excitatory neurotransmission (36), memory, learning (37) and long term potentiation (38-40). The role of glutamate in AD is well known, Memantine, an NMDA antagonist is used to treat

moderate to severe AD and recently it has been shown to have affinity for the dopamine receptors as well (41).

In this study GABA is increased in abundance but no change is seen in glutamate levels apart from a modest shift observed in the ITG. Whilst increased GABA production could still be coming at least in part from glutamate, the changes observed in other metabolites associated with GABA metabolism mean that the alterations may arise from multiple sources (Figure 2). GABA is the main inhibitory neurotransmitter in the mammalian nervous system (42, 43). It does not cross the blood brain barrier (44) and in the brain is predominantly synthesised from the non-essential amino acid glutamate by the action of glutamate decarboxylase under standard physiological conditions (45). However, GABA can be synthesised via several pathways from a selection precursors, including aminobutanal by aminobutyraldehyde dehydrogenase (46), succinate semialdehyde by aminobutyrate aminotransferase (47) and guanidinobutanoate by guanidinobutyrase (Supp Figure 1). Two alternative GABA synthetic pathways, both of which start from the urea cycle, have intermediates that are significantly reduced in abundance (Table 2, Supp Figure 1) suggesting that they may play a role in the dysregulation of GABA metabolism.

Regardless of the synthetic source of the increased abundance of GABA, this combined with the reduction in glutamate in the ITG (Table 2) can produce a reduction in the glutamate/GABA ratio, leading to a more inhibitory environment and a reduction in the transmission of action potentials. When GABA is released into the synaptic cleft it binds to a range of transmembrane receptors on both the pre and post-synaptic neurons leading to the opening of ion channels allowing the negatively charged chloride ions to enter and positively charged potassium ions to escape the neuron (Figure 2) (48). This shift leads to loss of the transmembrane potential and hyperpolarisation of the cell membrane, inhibiting action potentials produced by excitatory neurotransmitters like glutamate.

In conclusion, our results suggest that abnormalities in dopamine neurotransmission may be related to AD pathology and that combined therapeutic approaches, especially those affecting the GABAergic and serotonergic system might be useful as adjunctive treatments in AD.

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CONFLICT OF INTEREST

The authors report no conflicts of interest.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1 Boxplots showing the effect of disease status on the abundance of 6 neurotransmitters and 9 related metabolites in the Cerebellum, Inferior Temporal Gyrus and Middle Frontal Gyrus. Dysregulation of 5 neurotransmitters and 8 related metabolites shown by boxplots of three disease statuses separated by brain region A) tyrosine, B) DOPA C) dopamine D) aminobutanal E) arginine F) aspartate G) GABA H) glutamate I) glutamine J) guanosine K) ornithine M) serotonin N) tryptophan. DOPA; dihydroxyphenylalanine, GABA; gamma-aminobutyrate.

Supplemental Figure 2 Potential metabolic modifications of arginine metabolism and GABA production related to Alzheimer's pathogenesis. Metabolites shown in Red are decreased in abundance, blue are increased, black were measured but unchanged, and grey were non measured intermediates. ADase; Arginine deaminease, ARG; Arginase, ASL; Arginosuccinate lyase, ATase; Arginine transaminase, CKase; Creatine Kinase, GABA-T; GABA transaminase, GaMT – Guanidoacetate Methyltransferase Gase –Glutaminase GAT – Glycine amidinotransferase Gbase – Guanidobutyrase GBD – Guanidobutyraldehyde dehydrogenase GOD – Guanidino-oxopentanoate-decaroxylase GSase – Glutamine synthase OCT – Ornithine Carbomyltransferase ODC – Ornithine decarboxylase PAT – Putrescine Aminotransferase SSADH – Succinate semialdehyde dehydrogenase

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Tables

Table 1. Clinical Characteristics of study participants.

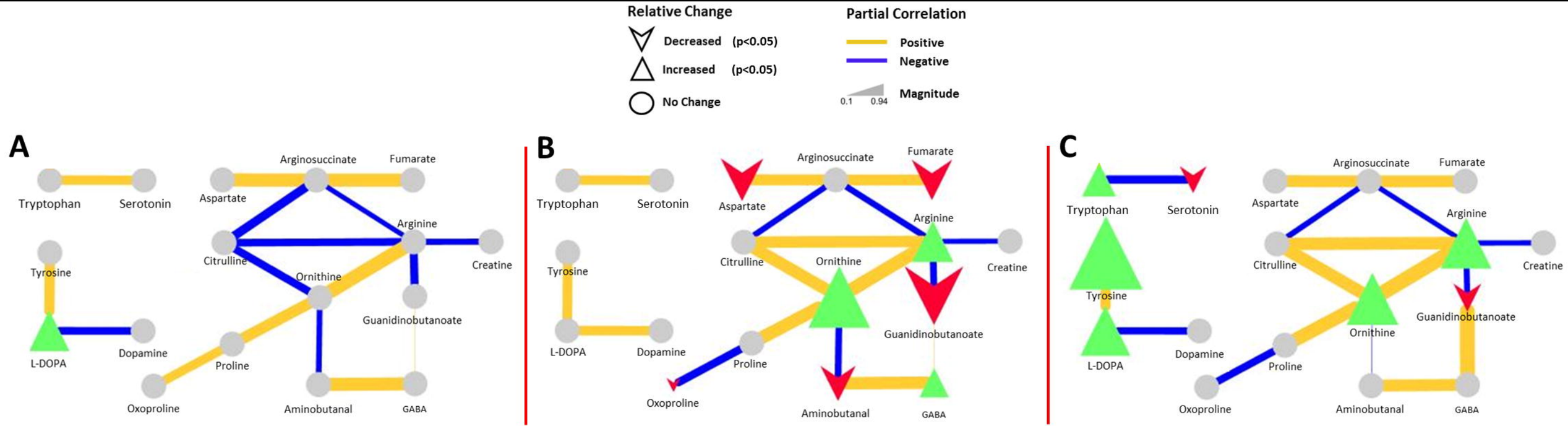
	Control	Asymptomatic	Demented
Participants (f/m)	14 (4/10)	15 (5/10)	14 (7/7)
Age at death (Years)[a]	82.6 +/- 11.0 (64.2-99.2)	89.2 +/- 7.9 (71.9-96.4)	87.9 +/- 8.9 (62.9-98.7)
MMSE[b]	27.8 +/- 2.4	29.0 +/- 0.9*	23.0 +/- 6.9**
PMI (Hours) [c]	16.9 +/- 6.4 (7.0-28.0)	14.8 +/- 8.1 (2.0-33.0)	14.7 +/- 6.0 (3.0-23.0)
Cholinesterase/NMDA usage	0/0	0/0	2/0

[a] values are reported as the mean +/- standard deviation, and range, [b] values are reported as the mean +/- standard deviation [c] values are reported as the mean +/- standard deviation, and range. MMSE: Mini-mental state examination, PMI: post mortem interval.

Table 2 Relative changes in abundance of 15 metabolites associated with neurotransmitter metabolism between all three diagnostic groups in individual brain regions.

	Cont vs Asymp						Cont vs Dem						Asymp vs Dem					
	Cerebellum		ITG (tau)		MFG (AB)		Cerebellum		ITG (tau)		MFG (AB)		Cerebellum		ITG (tau)		MFG (AB)	
	FC[a]	p[b]	FC[a]	p[b]	FC[a]	p[b]	FC[a]	p[b]	FC[a]	p[b]	FC[a]	p[b]	FC[c]	p[b]	FC[c]	p[b]	FC[c]	p[b]
Tyrosine	1.19	2.6×10^{-1}	1.21	1.5×10^{-1}	1.32	7.1×10^{-2}	1.17	2.3×10^{-1}	1.26	8.9×10^{-2}	**1.49**	**2.9×10^{-2}**	0.99	9.3×10^{-1}	1.04	7.8×10^{-1}	1.13	4.4×10^{-1}
L-DOPA	1.21	9.5×10^{-2}	1.06	5.5×10^{-1}	1.16	3.8×10^{-1}	**1.35**	**1.0×10^{-2}**	1.06	4.7×10^{-1}	**1.33**	**$4.4\times10^{-3*}$**	1.12	1.3×10^{-1}	1.00	9.5×10^{-1}	1.20	9.0×10^{-1}
Dopamine	0.98	9.0×10^{-1}	0.97	8.4×10^{-1}	**0.78**	**$4.1\times10^{-3*}$**	0.82	1.8×10^{-1}	0.88	2.9×10^{-1}	0.78	2.3×10^{-1}	0.83	2.1×10^{-1}	0.9	5.4×10^{-1}	0.99	9.6×10^{-1}
Tryptophan	0.99	9.4×10^{-1}	1.36	8.8×10^{-2}	1.24	1.8×10^{-1}	1.17	3.6×10^{-1}	**1.51**	**3.0×10^{-2}**	1.40	1.2×10^{-1}	1.18	3.0×10^{-1}	1.11	5.6×10^{-1}	1.12	5.3×10^{-1}
Serotonin	N/M	N/M	1.06	7.7×10^{-1}	0.79	1.0×10^{-1}	N/M	N/M	**0.64**	**4.4×10^{-2}**	0.93	6.9×10^{-1}	N/M	N/M	0.60	3.5×10^{-2}	1.18	3.4×10^{-1}
Aminobutanal	1.01	7.8×10^{-1}	**0.86**	**2.2×10^{-2}**	0.96	4.1×10^{-1}	0.94	2.7×10^{-1}	**0.76**	**$9.3\times10^{-5*}$**	0.93	1.5×10^{-1}	0.93	1.5×10^{-1}	0.89	1.5×10^{-1}	0.97	6.8×10^{-1}
Arginine	0.96	8.1×10^{-1}	1.38	8.3×10^{-2}	1.48	6.0×10^{-2}	1.1	5.1×10^{-1}	**1.59**	**$3.0\times10^{-3*}$**	**1.45**	**1.4×10^{-2}**	1.14	4.2×10^{-1}	1.15	4.2×10^{-1}	0.99	9.4×10^{-1}
Aspartate	1.01	9.3×10^{-1}	0.96	6.8×10^{-2}	1.06	6.6×10^{-1}	0.88	2.5×10^{-1}	**0.80**	**$2.7\times10^{-2*}$**	1.00	9.9×10^{-1}	0.87	2.4×10^{-1}	0.83	9.6×10^{-2}	0.95	5.8×10^{-1}
GABA	1.15	2.2×10^{-1}	1.16	1.3×10^{-1}	1.07	9.4×10^{-1}	1.06	6.0×10^{-2}	**1.23**	**$1.6\times10^{-2*}$**	1.16	5.0×10^{-2}	1.01	4.1×10^{-1}	1.07	4.5×10^{-1}	**1.23**	**$3.7\times10^{-2*}$**
Glutamate	1.05	4.1×10^{-1}	0.96	4.7×10^{-1}	0.99	8.1×10^{-1}	1.04	3.4×10^{-1}	**0.89**	**$9.9\times10^{-3*}$**	0.98	6.2×10^{-1}	0.99	8.3×10^{-1}	0.93	2.1×10^{-1}	0.99	8.4×10^{-1}
Glutamine	1.04	6.9×10^{-1}	0.98	8.1×10^{-1}	1.05	6.3×10^{-1}	1.04	6.2×10^{-1}	0.91	1.1×10^{-1}	1.04	6.6×10^{-1}	1.00	9.8×10^{-1}	0.92	3.2×10^{-1}	0.99	9.2×10^{-1}
Guanidinobutanoate	0.91	3.3×10^{-1}	0.88	1.3×10^{-1}	1.04	5.6×10^{-1}	0.82	6.0×10^{-2}	**0.61**	**$4.8\times10^{-5*}$**	**0.81**	**1.3×10^{-2}**	0.90	3.1×10^{-1}	**0.69**	**$1.2\times10^{-4*}$**	**0.78**	**4.3×10^{-2}**
Glycine	0.99	9.5×10^{-1}	1.12	3.9×10^{-1}	1.13	2.9×10^{-1}	0.99	8.0×10^{-1}	**0.83**	**$1.8\times10^{-2*}$**	0.95	6.1×10^{-1}	0.97	9.0×10^{-1}	0.74	5.1×10^{-2}	0.84	1.2×10^{-1}
Guanosine	0.98	9.5×10^{-1}	**0.72**	**1.7×10^{-2}**	0.87	4.1×10^{-1}	1.16	4.3×10^{-1}	**0.64**	**$3.2\times10^{-3*}$**	**0.70**	**1.9×10^{-2}**	1.17	4.9×10^{-1}	0.89	5.0×10^{-1}	0.80	2.6×10^{-1}
Ornithine	0.94	6.1×10^{-1}	1.27	1.2×10^{-1}	1.41	6.0×10^{-2}	1.04	6.9×10^{-1}	**1.41**	**$5.1\times10^{-3*}$**	**1.41**	**3.1×10^{-2}**	1.11	3.7×10^{-1}	1.11	4.8×10^{-1}	1.00	9.9×10^{-1}

[a] mean fold change relative to controls, [b] p-value calculated using mann-whitney U-test., [c] mean fold change relative to asymptomatic, [*] passes FDR using Benjamini-Hochberg correction (0.05 threshold), DOPA: dihydroxy-phenylalanine, GABA: gamma-aminobutanoate, ITG: Inferior Temporal Gyrus, MFG: Medial Frontal Gyrus.


Relative Change
Decreased (p<0.05)
Increased (p<0.05)
No Change
Partial Correlation
Positive
Negative
0.1
0.94
Magnitude
A
Tryptophan
Serotonin
Arginosuccinate
Fumarate
Aspartate
Arginine
Citrulline
Ornithine
Creatine
Tyrosine
Guanidinobutanoate
L-DOPA
Dopamine
Proline
Oxoproline
Aminobutanal
GABA
B
Tryptophan
Serotonin
Arginosuccinate
Fumarate
Aspartate
Arginine
Citrulline
Ornithine
Creatine
Tyrosine
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Dopamine
Proline
Oxoproline
Aminobutanal
GABA
C
Tryptophan
Serotonin
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Fumarate
Aspartate
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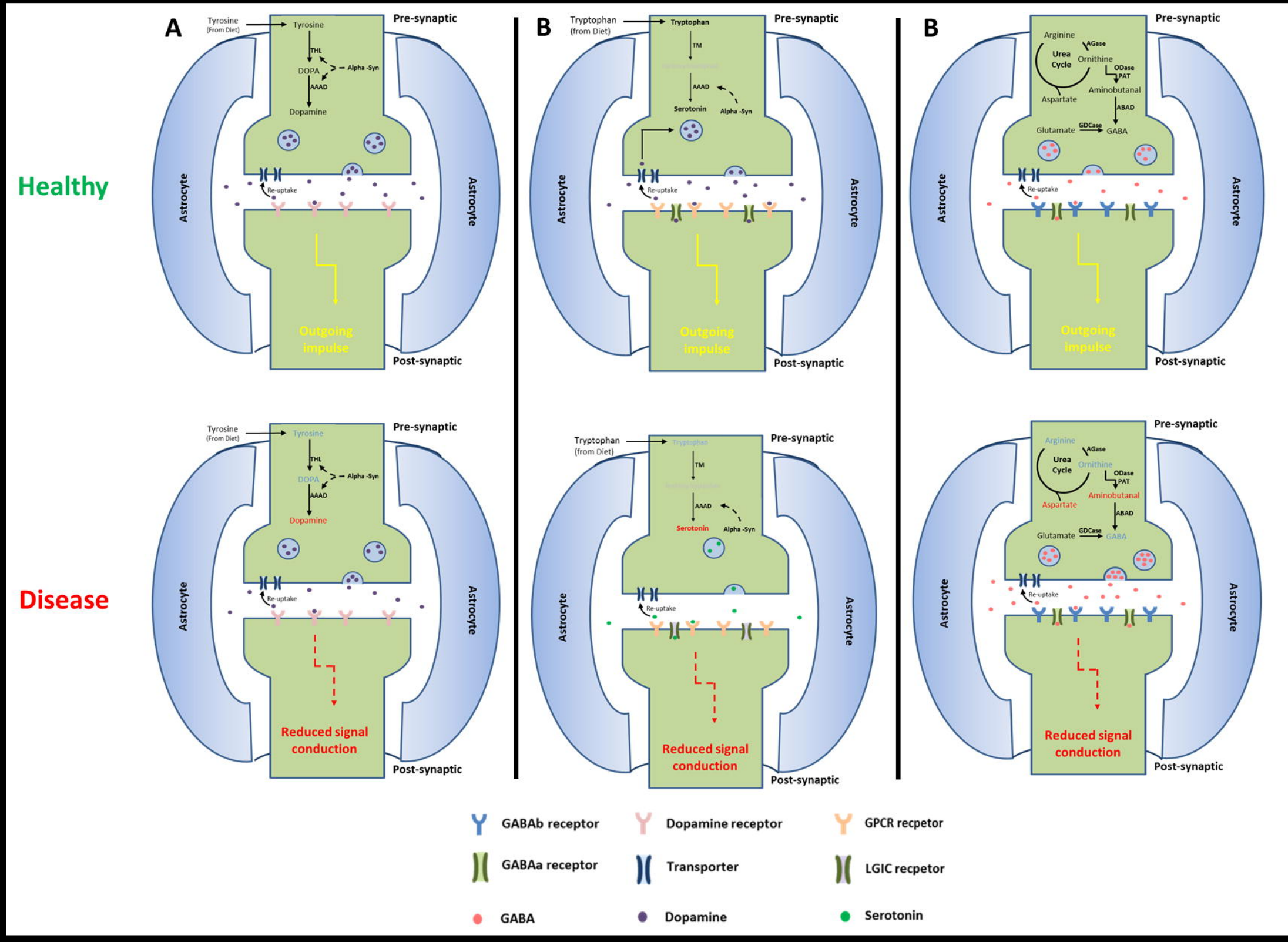
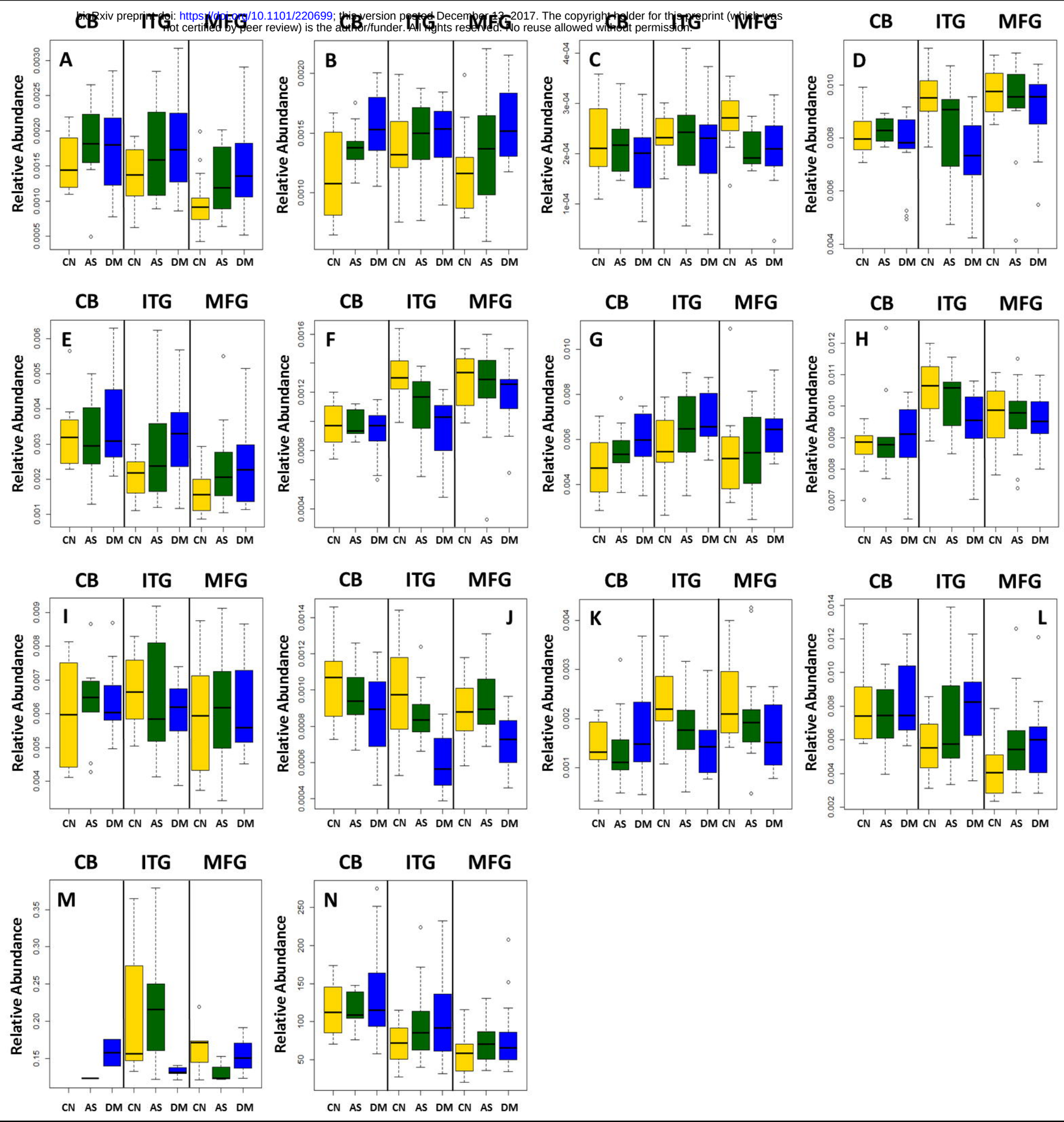
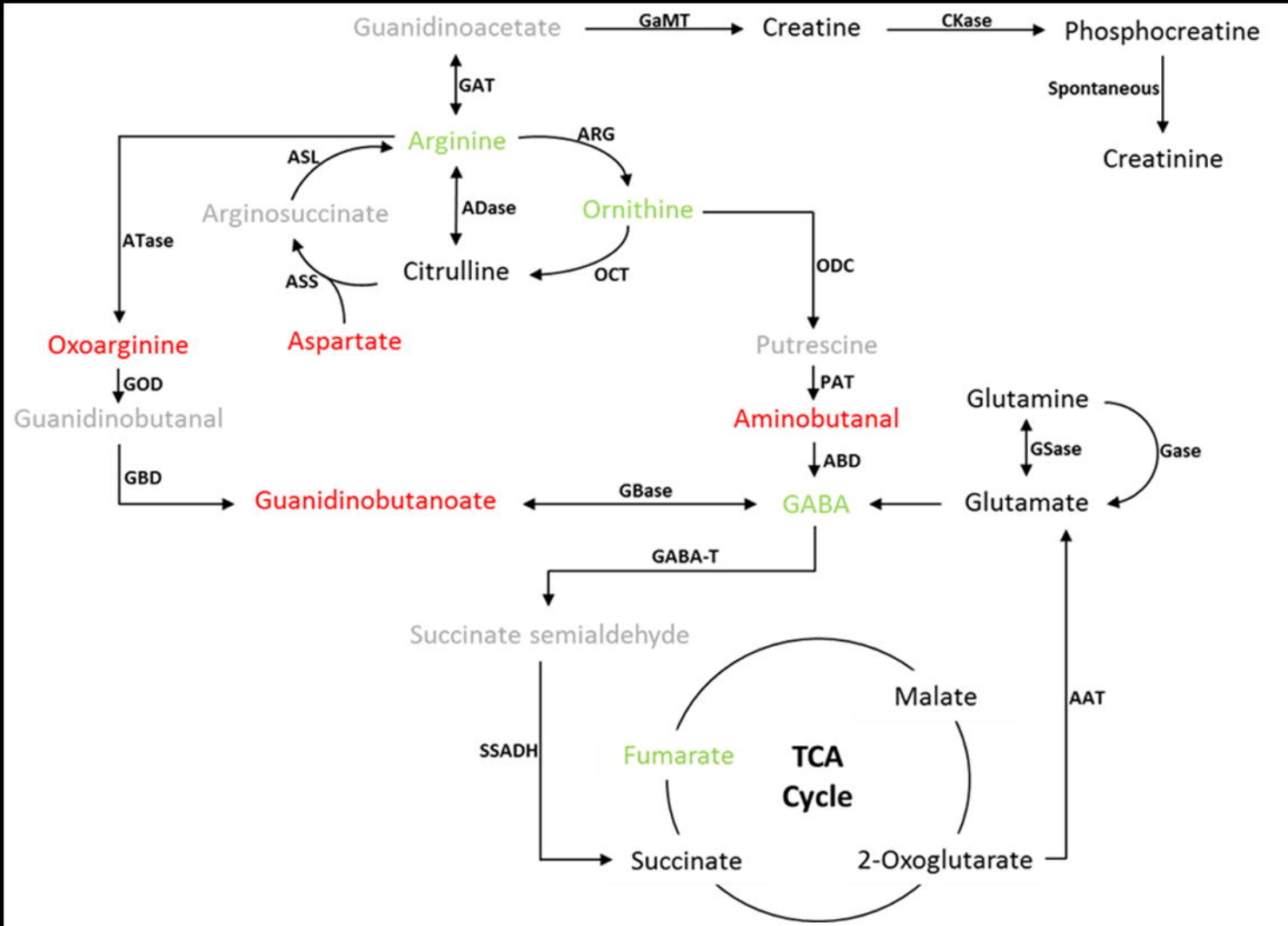

Healthy
Disease
A
B
B
Pre-synaptic
Post-synaptic
Astrocyte
Astrocyte
Tyrosine
(From Diet)
Tyrosine
THL
DOPA
Alpha -Syn
AAAD
Dopamine
Re-uptake
Outgoing impulse
Tryptophan
(from Diet)
Tryptophan
TM
AAAD
Serotonin
Alpha -Syn
Arginine
AGase
Urea Cycle
Ornithine
ODase
PAT
Aminobutanal
Aspartate
ABAD
Glutamate
GDCase
GABA
Reduced signal conduction
GABAb receptor
Dopamine receptor
GPCR recpetor
GABAa receptor
Transporter
LGIC recpetor
GABA
Dopamine
Serotonin




Guanidinoacetate
GaMT
Creatine
CKase
Phosphocreatine
Spontaneous
Creatinine
GAT
Arginine
ARG
ASL
Arginosuccinate
ADase
Ornithine
ATase
ODC
Citrulline
OCT
ASS
Aspartate
Oxoarginine
Putrescine
GOD
PAT
Guanidinobutanal
Aminobutanal
Glutamine
GSase
Gase
GBD
ABD
Guanidinobutanoate
GBase
GABA
Glutamate
GABA-T
Succinate semialdehyde
Malate
AAT
SSADH
Fumarate
TCA
Cycle
Succinate
2-Oxoglutarate