Supplementary Information

Bioanalytical Studies in the Brain of Wistar Rats (*Rattus norvegicus*) Exposed to 2,3-Butanedione

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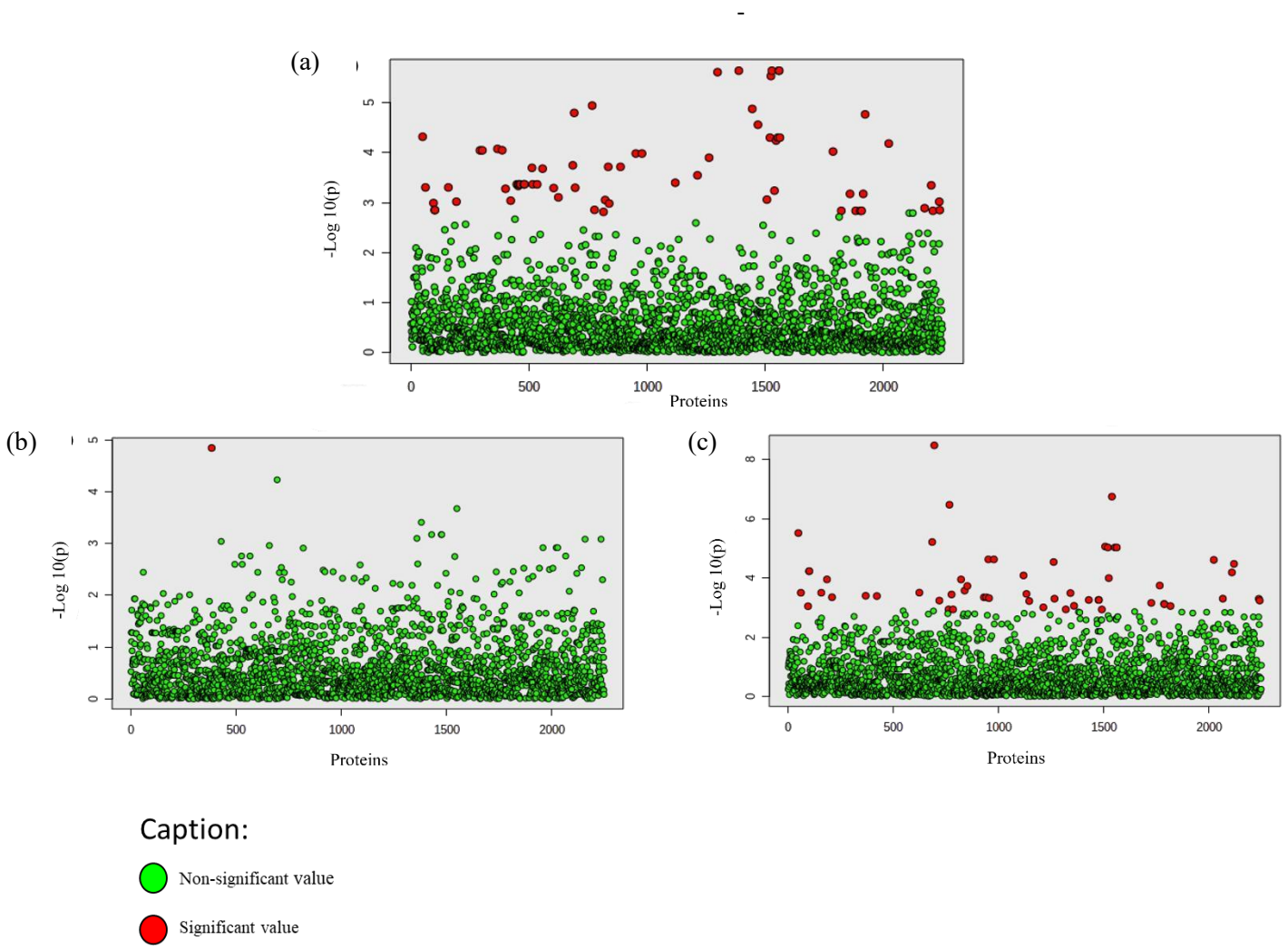
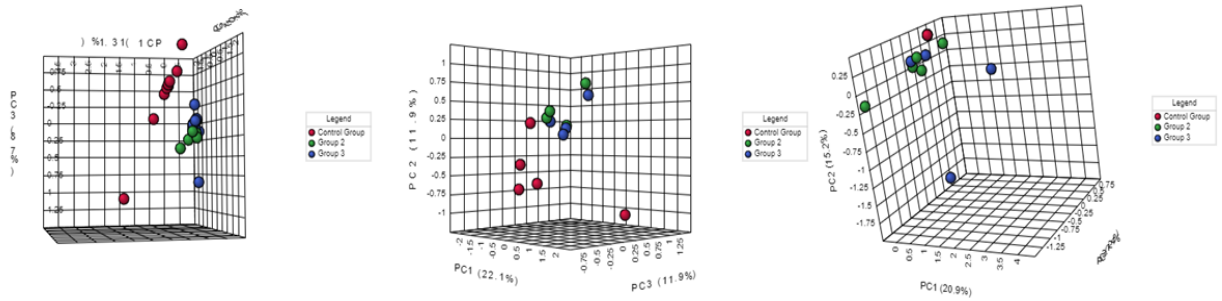


Figure S1. Amount of significant proteins found in brains after 2,3-butanedione exposure by analysis of variance (ANOVA, $p \leq 0.05$). (a) Without distinction of gender, (b) females, and (c) males.



(a) (b) (c)

Figure S2. Principal component analysis (PCA) obtained 3D graph. (a) Without distinction of gender, (b) females, and (c) males.

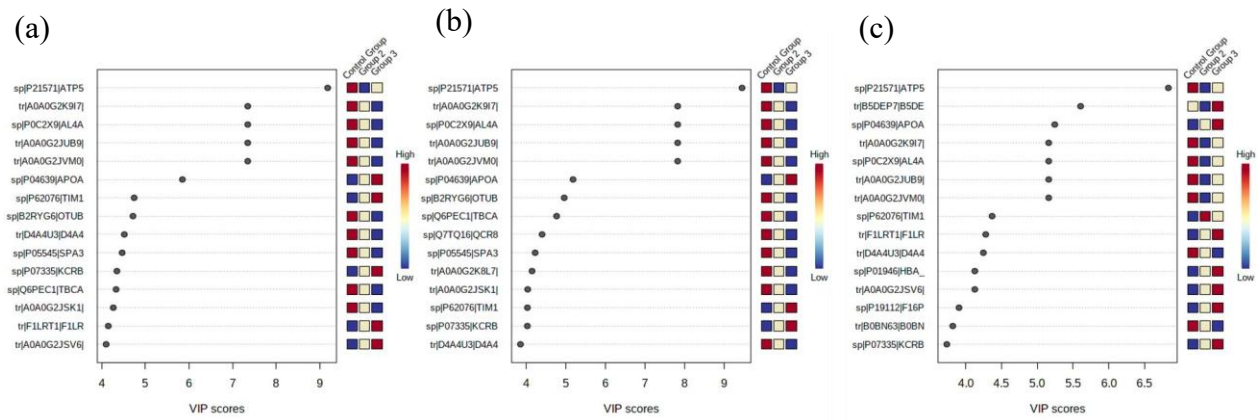


Figure S3. Fifteen highest variable importance in projection (VIP) scores obtained in the chemometric method used where (a) without gender distinction, (b) females and (c) males.

Table S1. Pathways and number of components identified in the model regardless of gender

Pathway names	Components
Gamma-aminobutyric acid synthesis	3
Nicotine pharmacodynamics pathway	27
CCKR signaling map	290
Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway	21
Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade	12
T cell activation	45
Methylcitrate cycle	5
Cysteine biosynthesis	2
Methylmalonyl pathway	3
Formyltetrahydrofolate biosynthesis	7
Serine glycine biosynthesis	4
N-Acetylglucosamine metabolism	9
Metabotropic glutamate receptor group I pathway	10
Alzheimer disease-amyloid secretase pathway	30
ATP synthesis	12
Beta1 adrenergic receptor signaling pathway	7
Folate biosynthesis	11
5HT1 type receptor mediated signaling pathway	8
FAS signaling pathway	31
Leucine biosynthesis	4
Adrenaline and noradrenaline biosynthesis	12
5HT2 type receptor mediated signaling pathway	9
Blood coagulation	59
Inflammation mediated by chemokine and cytokine signaling pathway	58
Endothelin signaling pathway	27
De novo purine biosynthesis	23
Thiamine metabolism	12
TGF-beta signaling pathway	20
Dopamine receptor mediated signaling pathway	27
Plasminogen activating cascade	22
Wnt signaling pathway	49
Apoptosis signaling pathway	72

Muscarinic acetylcholine receptors 1 and 3 signaling pathway	12
Opioid proopiomelanocortin pathway	9
B cell activation	37
VEGF signaling pathway	25
Oxytocin receptor mediated signaling pathway	6
Metabotropic glutamate receptor group II pathway	7
Methionine biosynthesis	4
Cortocotropin releasing factor receptor signaling pathway	9
Alpha adrenergic receptor signaling pathway	6
Interleukin signaling pathway	36
Pyruvate metabolism	10
Ionotropic glutamate receptor pathway	29
Insulin/IGF pathway-protein kinase B signaling cascade	18
Opioid prodynorphin pathway	12
Succinate to proprionate conversion	3
Pyrimidine metabolism	8
Gonadotropin releasing hormone receptor pathway	216
FGF signaling pathway	26
Xanthine and guanine salvage pathway	7
Ras pathway	39
5HT4 type receptor mediated signaling pathway	7
5HT3 type receptor mediated signaling pathway	5
Nicotinic acetylcholine receptor signaling pathway	13
Glycolysis	10
Huntington disease	60
Thyrotropin-releasing hormone receptor signaling pathway	9
Cytoskeletal regulation by Rho GTPase	20
Interferon-gamma signaling pathway	10
Fructose galactose metabolism	8
Purine metabolism	5
Cadherin signaling pathway	16
Metabotropic glutamate receptor group III pathway	12
Integrin signaling pathway	46
Ubiquitin proteasome pathway	6
PDGF signaling pathway	36
Aminobutyrate degradation	2

Angiotensin II-stimulated signaling through G proteins and beta-arrestin	21
Muscarinic acetylcholine receptor 2 and 4 signaling pathway	18
Toll receptor signaling pathway	46
Angiogenesis	77
Axon guidance mediated by semaphorins	14
Parkinson disease	37
Beta3 adrenergic receptor signaling pathway	4
Opioid proenkephalin pathway	11
5-Hydroxytryptamine degredation	2
EGF receptor signaling pathway	28

CCKR: cholecystokinin receptor; G-protein: guanine nucleotide-binding protein; Gq: G protein alpha q subunit; Go: G protein alpha o subunit; IGF: insulin-like growth factor; MAP: microtubule-associated protein; T: testosterone; ATP: adenosine triphosphate; Beta1: beta-1 adrenergic receptor; 5HT1: 5-hydroxytryptamine receptor 1 (serotonin receptor 1); FAS: Fas cell surface death receptor; 5HT2: 5-hydroxytryptamine receptor 2 (serotonin receptor 2); TGF-beta: transforming growth factor beta; Wnt: Wingless/Integrated signaling protein; B: B lymphocyte; VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; Ras: Rat sarcoma small GTPase; 5HT4: 5-hydroxytryptamine receptor 4 (serotonin receptor 4); 5HT3: 5-hydroxytryptamine receptor 3 (serotonin receptor 3); Rho: Ras homolog family GTPase; GTPase: guanosine triphosphatase; PDGF: Platelet-derived growth factor; II: Angiotensin II; EGF: Epidermal growth factor.

Table S2. Paths and number of components annotated in the model using only females

Pathway names	Components
Isoleucine biosynthesis	5
Gamma-aminobutyric acid synthesis	3
Muscarinic acetylcholine receptor 2 and 4 signaling pathway	11
Nicotine pharmacodynamics pathway	27
Toll receptor signaling pathway	46
CCKR signaling map	290
Angiogenesis	77
Axon guidance mediated by semaphorins	14
Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway	21
Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade	12
T cell activation	45
Parkinson disease	37
Cysteine biosynthesis	2
Methylmalonyl pathway	3
Cell cycle	12
5-Hydroxytryptamine degredation	2
EGF receptor signaling pathway	28
Heme biosynthesis	15
p53 pathway	70
Valine biosynthesis	4
Serine glycine biosynthesis	4
N-Acetylglucosamine metabolism	9
Metabotropic glutamate receptor group I pathway	10
Vitamin D metabolism and pathway	11
ATP synthesis	12
FAS signaling pathway	31
Inflammation mediated by chemokine and cytokine signaling pathway	58
De novo purine biosynthesis	23
Integrin signalling pathway	46
TGF-beta signaling pathway	20
Ubiquitin proteasome pathway	6
Dopamine receptor mediated signaling pathway	27
Aminobutyrate degradation	2
Angiotensin II-stimulated signaling through G proteins and beta-arrestin	21
De novo pyrmidine ribonucleotides biosythesis	10

Wnt signaling pathway	49
Apoptosis signaling pathway	72
B cell activation	37
VEGF signaling pathway	25
Methionine biosynthesis	4
Pyruvate metabolism	10
Ionotropic glutamate receptor pathway	29
De novo pyrimidine deoxyribonucleotide biosynthesis	10
Insulin/IGF pathway-protein kinase B signaling cascade	18
Pyrimidine metabolism	8
Succinate to proprionate conversion	3
Gonadotropin releasing hormone receptor pathway	216
FGF signaling pathway	26
Xanthine and guanine salvage pathway	7
Ras pathway	39
Salvage pyrimidine ribonucleotides	8
Glycolysis	10
Huntington disease	60
Nicotinic acetylcholine receptor signaling pathway	13
Alzheimer disease-presenilin pathway	70
Cytoskeletal regulation by Rho GTPase	20
Fructose galactose metabolism	8
Purine metabolism	5
Cadherin signaling pathway	16
Metabotropic glutamate receptor group III pathway	12

CCKR: cholecystokinin receptor; G-protein: guanine nucleotide-binding protein; Gq: G protein alpha q subunit; Go: G protein alpha o subunit; IGF: insulin-like growth factor; MAP: microtubule-associated protein; T: testosterone; EGF: epidermal growth factor; p53: tumor protein p53; *N*-acetylglucosamine: *N*-acetyl-D-glucosamine; I: insulin; D: dopamine; ATP: adenosine triphosphate; FAS: Fas cell surface death receptor; TGF-beta: transforming growth factor beta; II: angiotensin II; Wnt: Wingless/Integrated signaling protein; B: B lymphocyte; VEGF: vascular endothelial growth factor; FGF: fibroblast growth factor; Ras: rat sarcoma small GTPase; Rho: Ras homolog family GTPase; GTPase: guanosine triphosphatase.

Table S3. List of components annotated to Alzheimer's only in female model

Alzheimer’s components found
Ire-1 N-terminal fragment
C99
LRP C-terminal fragment Erb-B4
Transmembrane fragment actin
Nicastrin
Unfolded protein
E-cadherin C-terminal fragment
E-cadherin
Frizzled
Notch C-terminal fragment
CD44 intracellular fragment NICD
CD44 C-terminal fragment
Capacitative calcium channels
Ire-1 C-terminal fragment
Fe65
APP
Erb-B4
Presenilin
Matrix metalloprotease
HAC1
Dsh alpha-catenin
Nectin 1alpha transmembrane fragment
Notch transmembrane fragment
Abeta
Tip60
Erb-B4 intracellular fragment
E-cadherin transmembrane fragment
Presenilin N-terminal fragment
Membrane metalloprotease
Wnt
TCF/LEF
Ire-1
CD44
Afadin
TACE
Erb-B4 N-terminal fragment
Pen-2
LRP
APPbeta

LRP transmembrane fragment gammacatenin

Notch fragment Presenilin C-terminal fragment

beta-catenin Furin

CSL

Nectin 1alpha intracellular fragment

Nectin 1alpha

Proteasome

Pro-BACE-1

Erb-B4 C-terminal fragment

Nectin 1alpha N-terminal fragment

E-cadherin N-terminal fragment

AICD

CD44 transmembrane fragment

E-cadherin intracellular fragment

LRP N-terminal fragment

Aph-1

Notch N-terminal fragment

BACE-1

LRP intracellular fragment

CD44 N-terminal fragment

GSK-3beta

Notch

Nectin 1alpha C-terminal fragment

BACE-1 pro-domain

Notch extracellular fragment

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e-1: Enhancer-1; C99: C-terminal fragment 99 of amyloid precursor protein; LRP: low-density lipoprotein receptor-related protein; Erb-B4: Erb-B2 receptor tyrosine kinase 4 (HER4); Fe65: brain protein Fe65 (APP intracellular domain-binding protein); APP: amyloid precursor protein; HAC1: homologous to ATF/CREB1 transcription factor 1; Dsh: Dishevelled protein; Abeta: amyloid beta peptide; Tip60: Tat-interactive protein 60 kDa (histone acetyltransferase KAT5); Wnt: Wingless-type MMTV integration site family protein; TCF/LEF: T-cell factor / Lymphoid enhancer-binding factor; CD44: Cluster of differentiation 44; Afadin: actin filament-binding protein Afadin (AFDN); TACE: TNF-alpha converting enzyme (ADAM17); Pen-2: Presenilin enhancer 2; APPbeta: beta-secretase-cleaved amyloid precursor protein fragment; CSL: CBF1/Su(H)/Lag-1 transcription factor complex; Pro-BACE-1: Pro-beta-site amyloid precursor protein cleaving enzyme 1; AICD: amyloid precursor protein intracellular domain; Aph-1: anterior pharynx defective 1; BACE-1: Beta-site amyloid precursor protein cleaving enzyme 1; GSK-3beta: glycogen synthase kinase-3 beta.

Table S4. Paths and number of components identified in the model using only males

Pathway names	Components
Axon guidance mediated by Slit/Robo	14
CCKR signaling map	290
Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pathway	21
T cell activation	45
Insulin/IGF pathway-mitogen activated protein kinase kinase/MAP kinase cascade	12
Methylcitrate cycle	5
Cysteine biosynthesis	2
Methylmalonyl pathway	3
P38 MAPK pathway	36
Histamine H1 receptor mediated signaling pathway	5
Beta1 adrenergic receptor signaling pathway	7
5HT1 type receptor mediated signaling pathway	8
Adenine and hypoxanthine salvage pathway	10
Adrenaline and noradrenaline biosynthesis	12
Leucine biosynthesis	4
5HT2 type receptor mediated signaling pathway	9
Blood coagulation	59
Inflammation mediated by chemokine and cytokine signaling pathway	58
Endothelin signaling pathway	27
De novo purine biosynthesis	23
Thiamine metabolism	12
TGF-beta signaling pathway	20
Dopamine receptor mediated signaling pathway	27
Plasminogen activating cascade	22
Axon guidance mediated by netrin	11
PI3 kinase pathway	35
P53 pathway feedback loops 2	32
Apoptosis signaling pathway	72
Wnt signaling pathway	49
Heterotrimeric G-protein signaling pathway-rod outer segment phototransduction	15
Muscarinic acetylcholine receptor 1 and 3 signaling pathway	12
Opioid proopiomelanocortin pathway	9
B cell activation	37
VEGF signaling pathway	25

Oxytocin receptor mediated signaling pathway	6
Metabotropic glutamate receptor group II pathway	7
Anandamide degradation	1
Methionine biosynthesis	4
Cortocotropin releasing factor receptor signaling pathway	9
Alpha adrenergic receptor signaling pathway	6
Interleukin signaling pathway	36
Pyruvate metabolism	10
Ionotropic glutamate receptor pathway	29
De novo pyrimidine deoxyribonucleotide biosynthesis	10
Vitamin B6 metabolism	19
Succinate to proprionate conversion	3
Opioid prodynorphin pathway	12
Gonadotropin releasing hormone receptor pathway	216
FGF signaling pathway	26
Ras pathway	39
5HT4 type receptor mediated signaling pathway	7
5HT3 type receptor mediated signaling pathway	5
Salvage pyrimidine ribonucleotides	8
Nicotinic acetylcholine receptor signaling pathway	13
Glycolysis	10
Huntington disease	60
Thyrotropin-releasing hormone receptor signaling pathway	9
Cytoskeletal regulation by Rho gtpase	20
Interferon-gamma signaling pathway	10
Fructose galactose metabolism	8
Cadherin signaling pathway	16
Metabotropic glutamate receptor group III pathway	12
Integrin signalling pathway	46
PLP biosynthesis	7
Ubiquitin proteasome pathway	6
PDGF signaling pathway	36
Angiotensin II-stimulated signaling through G proteins and beta-arrestin	21
De novo pyrmidine ribonucleotides biosythesis	10
Heterotrimeric G-protein signaling pathway-Gi alpha and Gs alpha mediated pathway	27
Beta2 adrenergic receptor signaling pathway	7
Muscarinic acetylcholine receptor 2 and 4 signaling pathway	11

Toll receptor signaling pathway	46
Angiogenesis	77
Axon guidance mediated by semaphorins	14
Parkinson disease	37
Beta3 adrenergic receptor signaling pathway	4
Opioid proenkephalin pathway	11
5-Hydroxytryptamine degredation	2
Cell cycle	12
EGF receptor signaling pathway	28
Formyltetrahydrofolate biosynthesis	7
Metabotropic glutamate receptor group I pathway	10
Serine glycine biosynthesis	4
N-acetylglucosamine metabolism	9
Alzheimer disease-amyloid secretase pathway	31
ATP synthesis	12
Folate biosynthesis	11
FAS signaling pathway	31

Slit/Robo: Slit ligand / Roundabout receptor signaling pathway; CCKR: cholecystokinin receptor; G-protein: guanine nucleotide-binding protein; Gq: Gq alpha subunit of heterotrimeric G protein; Go: Go alpha subunit of heterotrimeric G protein; T: testosterone; IGF: insulin-like growth factor; MAP: Mitogen-activated protein; P38 MAPK: p38 mitogen-activated protein kinase; H1: histamine receptor H1; Beta1: Beta-1 adrenergic receptor; 5HT1: 5-hydroxytryptamine receptor 1 (serotonin receptor 1); 5HT2: 5-hydroxytryptamine receptor 2 (serotonin receptor 2); TGF-beta: transforming growth factor beta; PI3: phosphoinositide 3-kinase; P53: tumor protein p53; Wnt: Wingless-type MMTV integration site family protein; B: vitamin B; VEGF: vascular endothelial growth factor; II: Angiotensin II; B6: vitamin B6 (pyridoxine); FGF: fibroblast growth factor; Ras: Rat sarcoma small GTPase; 5HT4: 5-hydroxytryptamine receptor 4 (serotonin receptor 4); 5HT3: 5-hydroxytryptamine receptor 3 (serotonin receptor 3); Rho: Ras homolog family small GTPase; PDGF: platelet-derived growth factor; Gi: Gi alpha subunit of heterotrimeric G protein; Gs: Gs alpha subunit of heterotrimeric G protein; Beta2: Beta-2 adrenergic receptor; Beta3: Beta-3 adrenergic receptor; EGF: epidermal growth factor; PLP: pyridoxal 5′-phosphate; ATP: adenosine triphosphate; FAS: Fas cell surface death receptor.