Basic theory of MANOVA, LDA and LOO methods

Because of the statistical methods are classical methods that have been widely used in data analyses, their description here is brief. For MANOVA, it is an extension of the univariate analysis of variance. This method is used in the cases where there are two or more dependent variables.

LDA is a classical pattern recognition method and can provide a classification model based on the combination of variables to predict a category or group to which a material belongs (Fisher 1936). The basic theory of LDA is to classify the dependent by dividing a n-dimensional parameters space into two regions. That space is separated by a hyperplane defined by a linear discriminant function as follows:

$$Y = a_0 + a_1 X_1 + a_2 X_2 + \cdots + a_n X_n$$

Where Y is a discriminant score, that is the dependant variable; $X_1, \ldots X_n$ represents the specific descriptor (in this case, metabolite levels); and $a_0 \ldots a_n$ are the weights of the corresponding parameters. The two regions formed by the hyperplane correspond to the two classes. Once the model was constructed, it is important to validate the predictabilit of that model. The simplest and a commonly used method of crossvalidation is the LOO method. In this method, the learning algorithm is trained multiple times, using all but one of the training set data points. At last, the mean error over all data points was calculated.

Table 1S: Mass and isotope balance equations for the metabolic calculation:

Mass balance equations:

$$dGlc_{brain}/dt = V_{\max in} Glc_{blood} / \left(K_M + Glc_{blood}\right) - V_{\max out} Glc_{brain} / \left(K_{Mout} + Glc_{brain}\right) - CMR_{glc}$$

$$d(\mathrm{Glu}_N)/dt = d(\mathrm{Glu}_A)/dt = d(\mathrm{KG}_N)/dt = d(\mathrm{KG}_A)/dt = d(\mathrm{Asp}_N)/dt = d(\mathrm{Asp}_A)/dt$$
$$= d(\mathrm{OAA}_N)/dt = d(\mathrm{OAA}_A)/dt = 0$$

Isotopic balance equations:

$$d(\mathrm{Glc}_{brain,C_1}{}^{*})/dt = V_{\mathbf{max}in}(\mathrm{Glc}_{blood,C_1^*})/(Km_{in} + \mathrm{Glc}_{blood}) - V_{\mathbf{max}out}(\mathrm{Glc}_{brain,C_1^*})/(Km_{out} + \mathrm{Glc}_{brain})$$
$$- CMR_{glc}(\mathrm{Glc}_{brain,C_1^*} / \mathrm{Glc}_{brain})$$

$$d(\mathrm{L}_3{}^{*})/dt = CMR_{\mathrm{Glc}}(\mathrm{Glc}_{brain,C1^*} / \mathrm{Glc}_{brain}) + V_{dilLac}(0) - (V_{pdh_N} + V_{pdh_A} + V_{pc} + V_{dil_{Lac}} + V_{pdh_{GABA}})(\mathrm{L}_3{}^{*} / \mathrm{L})$$

$$d(\mathrm{Glu}_{N_4}{}^{*})/dt = V_{cycle}(\mathrm{Gln}_4{}^{*} / \mathrm{Gln}) + V_X(\mathrm{KG}_{N_4}{}^{*} / \mathrm{KG}_N) - (V_{cycle} + V_X)(\mathrm{Glu}_{N_4}{}^{*} / \mathrm{Glu}_N)$$

$$d(\mathrm{Glu}_{A_4}{}^{*})/dt = V_{cycle}(\mathrm{Glu}_{N_4}{}^{*} / \mathrm{Glu}_{\mathrm{N}}) + (V_X + V_{ana})(\mathrm{KG}_{A_4}{}^{*} / \mathrm{KG}_A) - (V_{\mathrm{Gln}} + V_X)(\mathrm{Glu}_{A_4}{}^{*} / \mathrm{Glu}_A)$$

$$d(\mathrm{Gln}_4{}^{*})/dt = V_{\mathrm{Gln}}(\mathrm{Glu}_{A_4}{}^{*} / \mathrm{Glu}_{\mathrm{A}}) + V_{dil\mathrm{Gln}}(0) - (V_{\mathrm{cycle}} + V_{efflux} + V_{\mathrm{cycle\ GABAgln}})(\mathrm{Gln}_4{}^{*} / \mathrm{Gln})$$

$$d(\mathrm{KG}_{A_4}{}^{*})/dt = V_{\mathrm{pdh_A}}(\mathrm{L}_3{}^{*} / \mathrm{L}) + V_X(\mathrm{Glu}_{A_4}{}^{*} / \mathrm{Glu}_{\mathrm{A}}) - (V_{\mathrm{TCA_A Net}} + V_X)(\mathrm{KG}_{A_4}{}^{*} / \mathrm{KG}_A)$$

$$d(\mathrm{KG}_{A_3}{}^{*})/dt = V_X(\mathrm{Glu}_{A_3}{}^{*} / \mathrm{Glu}_{\mathrm{A}}) + V_{TCA_A}(\mathrm{OAA}_{A_2}{}^{*} / \mathrm{OAA}_{\mathrm{A}}) - (V_{\mathrm{TCA_A Net}} + V_X)(\mathrm{KG}_{A_3}{}^{*} / \mathrm{KG}_A)$$

$$d(\mathrm{Glu}_{A_3}{}^{*})/dt = V_{cycle}(\mathrm{Glu}_{N_3}{}^{*} / \mathrm{Glu}_N) + V_X(\mathrm{KG}_{A_3}{}^{*} / \mathrm{KG}_A) - (V_{\mathrm{Gln}} + V_X)(\mathrm{Glu}_{A_3}{}^{*} / \mathrm{Glu}_A)$$

$$d(\mathrm{Glu}_{N_3}{}^{*})/dt = V_{cycle}(\mathrm{Gln}_3{}^{*} / \mathrm{Gln}) + V_X(\mathrm{KG}_{N_3}{}^{*} / \mathrm{KG}_N) - (V_X + V_{cycle})(\mathrm{Glu}_{N_3}{}^{*} / \mathrm{Glu}_N)$$

$$d(\mathrm{Gln}_{A3}{}^{*})/dt = V_{\mathrm{Gln}}(\mathrm{Glu}_{A_3}{}^{*} / \mathrm{Glu}_A) + V_{dil\mathrm{Gln}}(0) - (V_{\mathrm{cycle}} + V_{efflux} + V_{\mathrm{cycle_{GABAGln}}})(\mathrm{Gln}_3{}^{*} / \mathrm{Gln})$$

$$d(\mathrm{KG}_{N_4}{}^{*})/dt = V_{\mathrm{TCA_N}}(\mathrm{L}_3{}^{*} / \mathrm{L}) + V_X(\mathrm{Glu}_{N_4}{}^{*} / \mathrm{Glu}_{\mathrm{N}}) + \mathrm{V}_{\mathrm{dil\ N}}(0) - (V_{\mathrm{TCA_N}} + V_X)(\mathrm{KG}_{N_4}{}^{*} / \mathrm{KG}_N)$$

$$d(\mathrm{KG}_{N_3}{}^{*})/dt = V_X(\mathrm{Glu}_{N_3}{}^{*} / \mathrm{Glu}_{\mathrm{N}}) + V_{TCA_N}(\mathrm{OAA}_{N_2}{}^{*} / \mathrm{OAA}_{\mathrm{N}}) - (V_{\mathrm{TCA_N}} + V_X)(\mathrm{KG}_{N_3}{}^{*} / \mathrm{KG}_N)$$

$$d(\mathrm{Asp}_{N3}{}^{*})/dt = V_{X_N\mathrm{OAAAsp}}[(\mathrm{OAA}_{N_3}{}^{*} / \mathrm{OAA}_N) - V_{X_N\mathrm{OAAAsp}}(\mathrm{Asp}_{N_3}{}^{*} / \mathrm{Asp}_N)]$$

$$d(\mathrm{Asp}_{A_3}{}^{*})/dt = V_{X_A\mathrm{OAAAsp}}[(\mathrm{OAA}_{A_3}{}^{*} / \mathrm{OAA}_{\mathrm{A}}) - V_{X_A\mathrm{OAAAsp}}(\mathrm{Asp}_{A_3}{}^{*} / \mathrm{Asp}_A)]$$

$$d(\mathrm{OAA}_{N_2}{}^{*})/dt = V_{\mathrm{X_N AspOAA}}(\mathrm{Asp}_{N_2}{}^{*} / \mathrm{Asp}_{\mathrm{N}}) + 0.5V_{\mathrm{TCA_N}}[(\mathrm{KG}_{N_4}{}^{*} / \mathrm{KG}_{\mathrm{N}}) + (\mathrm{KG}_{N_3}{}^{*} / \mathrm{KG}_N)]$$
$$-(V_{\mathrm{X}_N\mathrm{AspOAA}} + V_{\mathrm{TCA}_N})(\mathrm{OAA}_{N_2}{}^{*} / \mathrm{OAA}_N)$$

$$d(\mathrm{OAA}_{A_2}{}^{*})/dt = 0.5V_{TCA_A Net}(\mathrm{KG}_{A_4} / \mathrm{KG}_A + \mathrm{KG}_{A_3} / \mathrm{KG}_A) + V_{\mathrm{X_A AspOAA}}(\mathrm{Asp}_{A_2}{}^{*} / \mathrm{Asp}_A) + 0.5V_{PC}(\mathrm{L}_3 / \mathrm{L})$$
$$+ V_{\mathrm{cycle_{GABAGln}}}(\mathrm{GABA}_2 / \mathrm{GABA}) - (V_{\mathrm{X_A AspOAA}} + V_{\mathrm{TCA}_A})(\mathrm{OAA}_{A_2}{}^{*} / \mathrm{OAA}_A)$$

$$d(\mathrm{GABA}_2{}^{*})/dt = V_{gad}(\mathrm{Glu}_{GA_4}{}^{*} / \mathrm{Glu}_{GA}) - [V_{shunt} + V_{cycle_{GABAG\mathbf{ln}}}](\mathrm{GABA}_2{}^{*} / \mathrm{GABA})$$

$$d(\mathrm{KG}_{GA_4}{}^{*}/dt) = V_{pdh_{\mathrm{GABA}}}(\mathrm{L}_3{}^{*}/\mathrm{L}) + V_{X_{\mathrm{GGKG}}}(\mathrm{Glu}_{GA_4}{}^{*}/\mathrm{Glu}_{GA}) + V_{dil_{\mathrm{GG}}}(0)$$
$$- [V_{TCA_{\mathrm{GABA}}Net} + V_{X_{\mathrm{KGGG}}}](\mathrm{KG}_{GA_4}{}^{*}/\mathrm{KG}_{GA})$$

$$d(\mathrm{Glu}_{GA_4}{}^{*}/dt) = V_{cycle_{\mathrm{GABAGln}}}(\mathrm{Gln}_4{}^{*}/\mathrm{Gln}) + V_{X_{\mathrm{KGGG}}}(\mathrm{KG}_{GA_4}/\mathrm{KG}_{GA}) - [V_{gad} + V_{X_{\mathrm{GGKG}}}](\mathrm{Glu}_{GA_4}/\mathrm{Glu}_{GA})$$

$$d(\mathrm{KG}_{GA_3}{}^{*}/dt) = V_{X_{GGKG}}(\mathrm{Glu}_{GA_3}{}^{*}/\mathrm{Glu}_{GA}) + V_{TCA_{\mathrm{GABA}}}(\mathrm{OAA}_{GA_3}{}^{*}/\mathrm{OAA}_{GA})$$
$$- [V_{TCA_{\mathrm{GABA}}Net} + V_{X_{\mathrm{KGGG}}}](\mathrm{KG}_{GA_3}/\mathrm{KG}_{GA})$$

$$d(\mathrm{GABA}_3{}^{*})/dt = V_{gad}(\mathrm{Glu}_{GA_3}{}^{*}/\mathrm{Glu}_{GA}) - [V_{shunt} + V_{cycle_{\mathrm{GABAGln}}}](\mathrm{GABA}_3{}^{*}/\mathrm{GABA})$$

$$d(\mathrm{Glu}_{GA_3}{}^{*})/dt = V_{cycle_{\mathrm{GABAGln}}}(\mathrm{Gln}_3{}^{*}/\mathrm{Gln}) + V_{X_{\mathrm{KGGG}}}(\mathrm{KG}_{GA_3}{}^{*}/\mathrm{KG}_{GA}) - [V_{gad} + V_{X_{\mathrm{GGKG}}}](\mathrm{Glu}_{GA_3}{}^{*}/\mathrm{Glu}_{GA})$$

$$d(\mathrm{Asp}_{GA_3}{}^{*})/dt = V_{X_{\mathrm{GA}}\mathrm{OAAAsp}}(\mathrm{OAA}_{GA_2}{}^{*}/\mathrm{OAA}_{GA}) - V_{X_{\mathrm{GA}}\mathrm{AspOAA}}(\mathrm{Asp}_{GA_3}{}^{*}/\mathrm{Asp}_{GA})$$

$$d(\mathrm{OAA}_{N_3}{}^{*})/dt = V_{X_{\mathrm{AspOAA}}}(\mathrm{Asp}_{N_2}{}^{*}/\mathrm{Asp}_N) + 0.5V_{\mathrm{TCA}_N}(\mathrm{KG}_{N_3}{}^{*}/\mathrm{KG}_N) + 0.5V_{\mathrm{TCA}_N}(\mathrm{KG}_{N_4}{}^{*}/\mathrm{KG}_N)$$
$$- [V_{X_{\mathrm{OAAAsp}}N} + V_{\mathrm{TCA}_N}](\mathrm{OAA}_{N_3}{}^{*}/\mathrm{OAA}_N)$$

$$d(\mathrm{OAA}_{A_3}{}^{*})/dt = V_{cycle_{\mathrm{GABAGln}}}(\mathrm{GABA}_3/\mathrm{GABA}) + V_{X_{\mathrm{A}}\mathrm{AspOAA}}(\mathrm{Asp}_{A_2}{}^{*}/\mathrm{Asp}_A) + 0.5V_{pc}(\mathrm{L}_3{}^{*}/\mathrm{L}) + 0.5V_{\mathrm{TCA}Net}$$
$$(\mathrm{KG}_{A_4}{}^{*}/\mathrm{KG}_A) + 0.5V_{\mathrm{TCA}_ANet}(\mathrm{KG}_{A_3}{}^{*}/\mathrm{KG}_A) - [V_{X_{\mathrm{OAAAsp}}} + V_{\mathrm{TCA}_A}](\mathrm{OAA}_{A_3}/\mathrm{OAA}_A)$$

$$d(\mathrm{Asp}_{A_2}{}^{*})/dt = V_{X_A\mathrm{OAAAsp}}(\mathrm{OAA}_{A_2}{}^{*}/\mathrm{OAA}_A) - V_{X_A\mathrm{AspOAA}}(\mathrm{Asp}_{A_2}{}^{*}/\mathrm{Asp}_A)$$

$$d(\mathrm{Asp}_{N_2}{}^{*})/dt = V_{X_N\mathrm{OAAAsp}}(\mathrm{OAA}_{N_2}{}^{*}/\mathrm{OAA}_N) - V_{X_N\mathrm{AspOAA}}(\mathrm{Asp}_{N_2}{}^{*}/\mathrm{Asp}_N)$$

$$d(\mathrm{Asp}_{GA_2}{}^{*})/dt = V_{X_{GA}\mathrm{OAAAsp}}(\mathrm{OAA}_{GA_2}{}^{*}/\mathrm{OAA}_{GA}) - V_{X_{GA}\mathrm{AspOAA}}(\mathrm{Asp}_{GA_2}{}^{*}/\mathrm{Asp}_{GA})$$

$$d(\mathrm{OAA}_{GA_2}{}^{*})/dt = 0.5V_{TCA_{\mathrm{GABA}}Net}(\mathrm{KG}_{GA_3}{}^{*}/\mathrm{KG}_{GA}) + 0.5V_{TCA_{\mathrm{GABA}}Net}(\mathrm{KG}_{GA_4}{}^{*}/\mathrm{KG}_{GA}) + 0.5V_{shunt}$$
$$(\mathrm{GABA}_2{}^{*}/\mathrm{GABA}) + 0.5V_{shunt}(\mathrm{GABA}_3{}^{*}/\mathrm{GABA}) + V_{X_{GA}\mathrm{AspOAA}}(\mathrm{Asp}_{GA_2}{}^{*}/\mathrm{Asp}_{GA})$$
$$- [V_{TCA_{GABA}} + V_{X_{GA}OAAAsp}](OAA_{GA_2}{}^{*}/OAA_{GA})$$

$$d(\mathrm{OAA}_{GA_3}{}^{*})/dt = 0.5V_{TCA_{\mathrm{GABA}}Net}(\mathrm{KG}_{GA_4}{}^{*}/\mathrm{KG}_{GA}) + 0.5V_{TCA_{\mathrm{GABA}}Net}(\mathrm{KG}_{GA_3}{}^{*}/\mathrm{KG}_{GA}) + 0.5V_{shunt}$$
$$(\mathrm{GABA}_2{}^{*}/\mathrm{GABA}) + 0.5V_{shunt}(\mathrm{GABA}_3{}^{*}/\mathrm{GABA}) + V_{X_{GA}\mathrm{AspOAA}}(\mathrm{Asp}_{GA_3}{}^{*}/\mathrm{Asp}_{GA})$$
$$- [V_{TCA_{\mathrm{GABA}}} + V_{X_{GA}\mathrm{AspOAA}}](\mathrm{OAA}_{GA_3}{}^{*}/\mathrm{OAA}_{GA})$$

Combination Pools:

$\mathrm{Glu}_{4\_total} = \mathrm{Glu}_{A4} + \mathrm{Glu}_{N4} + \mathrm{Glu}_{GA4}$

$\mathrm{Glu}_{3\_total} = \mathrm{Glu}_{A3} + \mathrm{Glu}_{N3} + \mathrm{Glu}_{GA3}$

The following are one group of rates and concentrations from the parietal cortex in a saline-treated group using the upper equations and combinational pools.

Rates:

CMR_{gl} = ($Vpdh_A$+$Vpdh_N$+$Vpdh_{GABA}$+V_{pc})/2 = 0.54 µmol/min/g; *rate of glucose consumption*

K_{m_in} = 13.9 mM; *Michaelis-Menten half-saturation constant for blood-bran glucose Transport (Mason et al. 1992b)*

K_{m_out} = K_{m_in}*V_d = 10.70 µmol/g; *Michaelis-Menten half-saturation constant for bran-blood glucose transport*

$R\_{}_{AspGlu}$ = 0.29 µmol/min/g; *Fraction of Asp that is in glutamatergic neurons*

V_{cycle} = 0.49 µmol/min/g; *Rate of Glu-Gln cycling*

$V_{cycleGABAGln}$ = 0.46*$V_{tcaGABA}$ = 0.053 µmol/min/g; *Rate of GABA-Gln cycling, fraction of 0.46 from (Patel et al. 2005)*

V_d = 0.77 ml/g; *Brain water space (Buschiazzo et al. 1970)*

V_{dilGG} = 0.046 µmol/min/g; *Rate of dilution in GABAergic neurons*

V_{dilGln} = 0.35 µmol/min/g; *Rate of diluting Gln exchange with blood*

V_{dilN} = 0 µmol/min/g; *Rate of dilution in glutamatergic neurons*

V_{efflux} = V_{pc} + V_{dilGln} = 0.42 µmol/min/g; *Rate of loss of carbon from the astrocytic TCA cycle via efflux of Gln from the brain, partly balanced by entry of glutamine from the blood, also at the rate VdilGln.*

V_{gad} = V_{shunt}+$V_{cycleGABAGln}$ = 0.14 µmol/min/g; *Rate of GABA synthesis*

V_{gln} = V_{cycle}+$V_{cycleGABAGln}$+V_{pc} = 0.60 µmol/min/g*; Rate of Gln synthesis*

V_{max_in} = 5.8*CMR_{gl} = 3.14 µmol/min/g; *V_{max} for glucose flow from blood to brain (Mason et al. 1992b).*

V_{max_out} = V_{max_in} = 3.14 µmol/min/g; *Vmax for glucose flow from brain to blood*

V_{pc} = V_{tcaA}-$V_{tcaANet}$-$V_{cycleGABAGln}$ = 0.065 µmol/min/g; *Rate of anaplerosis*

V_{pdhA} = 0.176*V_{tcaN} = 0.14 µmol/min/g; *Rate of astrocytic pyruvate dehydrogenase (Patel et al. 2005)*

$V_{pdhGABA}$ = 0.070 µmol/min/g; *Rate of pyruvate dehydrogenase in the GABAergic neuron*

V_{shunt} = $V_{tcaGABA}$-$V_{tcaGABANet}$ = 0.088 µmol/min/g; *Rate of GABA degradation in the GABAergic neuron*

$V_{tcaGABA}$ = $V_{pdhGABA}$+V_{dilGG} = 0.12 µmol/min/g; *Rate of GABAergic TCA cycle from* *citric acid through KG*

$V_{tcaGABANet}$ = 0.029 μmol/min/g; *Rate of GABAergic TCA cycle flow from KG to succinate*

V_{tcaN} = 0.81 μmol/min/g; *Rate of glutamatergic neuronal TCA cycle rate*

$V_{xAspOAAGA} = V_{xOAAAspGA}$ = 0.5 μmol/min/g; *Rate of flow from Glu to KG in GABAergic neurons; value indeterminate*

V_{XGGKG} = 0.5 μmol/min/g; *Rate of flow from Glu to KG in astrocytes; value ndeterminate*

$V_{XKGGG} = V_{XGGKG} + V_{gad} - V_{cycleGABAGln}$ = 0.59 μmol/min/g; *Rate of flow from KG to Glu in astrocytes*

$V_{XOAAAspGA} = V_{XGGKG}$ = 0.5 μmol/min/g; *Rate of flow from OAA to Asp in GABAergic neurons*

$X_1 = V_{max\_in} * Glucose_{1blood}/(Km_{\_in} + Glucose_{1Blood})$ = 0.89 μmol/min/g; *Variable used to calculate steady-state brain glucose concentrations*

$X_2 = X_1 - CMR_{gl}$ = 0.35 μmol/min/g; *Variable used to calculate steady-state brain glucose concentrations*

V_{dilA} = 0.0058 μmol/min/g; *Rate of dilution in astrocytes*

$V_{tcaA} = V_{ac} + V_{pdhA} + V_{dilA}$ = 0.15 μmol/min/g; *Rate of TCA cycle in astrocytes*

$V_{tcaANet}$ = 0.029 μmol/min/g; *Rate of TCA from citric acid to KG in astrocytes*

$V_{XAAspOAA} = V_{XAOAAAsp}$ = 1 μmol/min/g; *Rate of flow from Asp to OAA in astrocytes*

$V_X = V_{XAGluKG}$ = 1 μmol/min/g; *Rate of flow from OAA to Asp in astrocytes*

$V_{XAKGGlu} = V_{XAGluKG} + V_{gln} - V_{cycle}$ = 1.12 μmol/min/g; *Rate of flow from KG to Glu in astrocytes*

$V_{XAOAAAsp} = V_{XAGluKG}$ = 1 μmol/min/g; *Rate of flow from OAA to Asp in astrocytes*

$V_{pdhN} = V_{tcaN} - V_{dilN}$ = 0.81 μmol/min/g; *Rate of flow through glutamatergic neuronal pyruvate dehydrogenase*

$V_{XNAspOAA} = V_{XNOAAAsp}$ = 3.64 μmol/min/g; *Rate of flow from Asp to OAA in glutamatergic neurons*

$V_{XNGluKG}$ = 3.64 μmol/min/g; *Rate of flow from Glu to KG in glutamatergic neurons*

$V_{XNKGGlu} = V_{XNGluKG}$ = 3.64 μmol/min/g; *Rate of flow from KG to Glu in glutamatergic neurons*

$V_{XNOAAAsp} = V_{XNGluKG}$ = 3.64 μmol/min/g; *Rate of flow from OAA to Asp in* *glutamatergic neurons*

Pool Concentrations:

$Asp_{GA} = Asp_{Total}$-Asp_A-Asp_N = 1.43 µmol/g; *GABAergic neuronal Asp*

Asp_{Total} = 3.16 µmol/g; *Total tissue Asp (measured)*

$Glucose_{brain} = Km_{\_out}{*}X_2/(V_{max\_out} - X_2)$ = 1.33 µmol/g; *Brain glucose (Mason et al. 1992a)*

GABA = 1.29 µmol/g; *GABA concentration (measured)*

Gln = 6.52 µmol/g; *Gln concentration (measured)*

$Glu_{GA} = 0.02{*}Glu_{Total}$ = 0.21 µmol/g; *Glu concentration in GABAergic neurons (small but indeterminate concentration)*

Glu_{Total} = 10.48 µmol/g; *Total tissue Glu (measured)*

KG_{GA} = 0.01 µmol/g; *KG in GABAergic neurons, estimated from (Hawkins & Mans 1983)*

L = 1.5 µmol/g; *Tissue lactate concentration (Hawkins & Mans 1983)*

OAA_{GA} = 0.05 µmol/g; *OAA concentration in GABAergic neurons, estimated from (Hawkins & Mans 1983)*

OAA_{Total} = 0.2 µmol/g; *Total tissue OAA (Hawkins & Mans 1983)*

$Asp_A = R\_AspGlu{*}Glu_A$ = 0.30 µmol/g; *Asp in astrocytes*

$Glu_A = 0.1{*}Glu_{Total}$ = 1.05 µmol/g; *Glu in astrocytes (Patel et al. 2010, Lebon et al. 2002)*

KG_A = 0.09 µmol/g; *KG in astrocytes, estimated from (Hawkins & Mans 1983)*

$OAA_A = OAA_{Total}$-OAA_N-OAA_{GA} = 0.05 µmol/g; *OAA in astrocytes, estimated from (Hawkins & Mans 1983)*

$Asp_N = 0.5{*}(Asp_{Total}$-$Asp_A)$ = 1.43 µmol/g; *Asp in glutamatergic neurons*

$Glu_N = Glu_{Total} - Glu_A - Glu_{GA}$ = 9.22 µmol/g; *Glu in glutamatergic neurons (Patel et al. 2010, Lebon et al. 2002)*

KG_N = 0.1 µmol/g; *KG in glutamatergic neurons, estimated from (Hawkins & Mans 1983)*

OAA_N = 0.1 µmol/g; *OAA in glutamatergic neurons, estimated from (Hawkins & Mans 1983)*

Note: Glc, glucose; L, lactate; Glu, glutamate; Gln, glutamine; Asp, aspartate; OAA, oxaloacetate; KG, α-ketoglutarate; Subscript 'N', 'A' and 'GA' stand for glutamatergic neuron, astrocytes and

GABAergic neurons, respectively. Numeric subscripts represent the position of ^{13}C in the corresponding molecule. Parameters listed in bold-faced type were determined by iterative fitting.

Table 2S. Mass of tissue samples (mg) from each region across all the rats.

No.	OB	CE	MEA	THA	HYP	HP	FC	PC	OC	ST	TC	MID
Mean	65.6	217.5	182.8	92.4	65.5	110.1	153.7	152.2	108.4	151.1	195.8	98.2
SD	9.7	21.7	21.4	12.7	15.5	10.7	23.1	22.3	14.0	30.9	23.5	12.5

Table 3S. The descriptors selected by the linear discriminant analysis and their corresponding statistical parameters for the whole group. Their selection was based on the ability to differentiate rats that received nicotine from those that received saline.

Region	Metabolite	Tolerance	F to remove	Wilks' Lambda	CDF
ST	GABA	0.7235	9.7047	0.4500	0.7563
ST	NAA	0.6113	20.4191	0.5890	1.0432
THA	GABA	0.5524	10.6467	0.4622	-0.8944
HYP	Glu	0.4518	11.3550	0.4714	1.0114
TC	GABA	0.6021	7.8397	0.4258	-0.7659

Note: CDF: Standardized Canonical Discriminant Function Coefficients.

Table 4S: Effect of nicotine on cerebral cortical metabolic fluxes (µmol·g·min^{-1}), including V_X (exchange rate between KG and glutamate in astrocytes), V_{dilGln} (the diluting inflow rate of glutamine), V_{tcaN} (TCA cycle flux in neuron), V_{dil} (total diluting flow into KG),

	V_X		V_{tcaN}		V_{dilGln}		V_{dil}	
Brain	Saline	Nicotine	Saline	Nicotine	Saline	Nicotine	Saline	Nicotine
CE	0.59±0.01	0.59±0.01	0.64±0.03	0.63±0.02	0.33±0.04	0.32±0.03	0.08±0.05	0.03±0.04
FC	0.57±0.01	0.57±0.00	0.77±0.05	0.66±0.02	0.30±0.04	0.27±0.02	0.02±0.04	0.01±0.02
TC	0.58±0.01	0.57±0.01	0.68±0.03	0.59±0.03	0.38±0.03	0.33±0.03	0.01±0.03	0.01±0.03
HP	0.58±0.01	0.57±0.01	0.64±0.03	0.52±0.02	0.37±0.04	0.30±0.03	0.00±0.04	0.02±0.03
HYP	0.58±0.01	0.57±0.01	0.53±0.02	0.47±0.02	0.36±0.03	0.36±0.03	0.01±0.03	0.01±0.03
MEA	0.59±0.01	0.57±0.00	0.53±0.02	0.47±0.02	0.39±0.03	0.34±0.03	0.00±0.03	0.02±0.03
MID	0.61±0.01	0.60±0.01	0.60±0.03	0.54±0.02	0.35±0.03	0.31±0.03	0.02±0.03	0.00±0.02
OB	0.56±0.01	0.57±0.00	0.58±0.04	0.52±0.03	0.33±0.04	0.34±0.04	0.02±0.05	0.02±0.04
OC	0.59±0.01	0.58±0.01	0.67±0.05	0.62±0.03	0.36±0.03	0.30±0.02	0.07±0.02	0.01±0.02
PC	0.59±0.01	0.57±0.01	0.81±0.03	0.67±0.03	0.35±0.03	0.26±0.02	0.01±0.03	0.00±0.02
ST	0.58±0.01	0.55±0.01	0.67±0.02	0.55±0.02	0.30±0.03	0.28±0.02	0.00±0.03	0.01±0.02
THA	0.58±0.01	0.58±0.00	0.64±0.03	0.65±0.03	0.33±0.03	0.33±0.03	0.01±0.02	0.00±0.02

Table 5S: The p-values of pair-wise comparisons of rates of metabolism after nicotine injection in different regions, with indications of which rates survived adjustments for multiple comparisons.

	CE	FC	FDC	HP	HYP	MEA	MID	OB	OC	PC	ST	THA
$CMR_{gl(ox)}$	0.449	0.007	0.045	0.010	0.031	0.038	0.022	0.008	**0.000***	**0.000***	0.004	0.433
V_{cycle}	0.196	0.212	0.238	0.303	0.522	0.742	0.153	0.313	**0.001***	**0.000***	0.267	0.537
V_{gad}	0.578	0.109	0.427	0.314	0.096	0.035	0.354	0.883	0.037	0.039	**0.025***	0.717

Note: *: The rates that remained significant after adjustment for multiple comparisons.

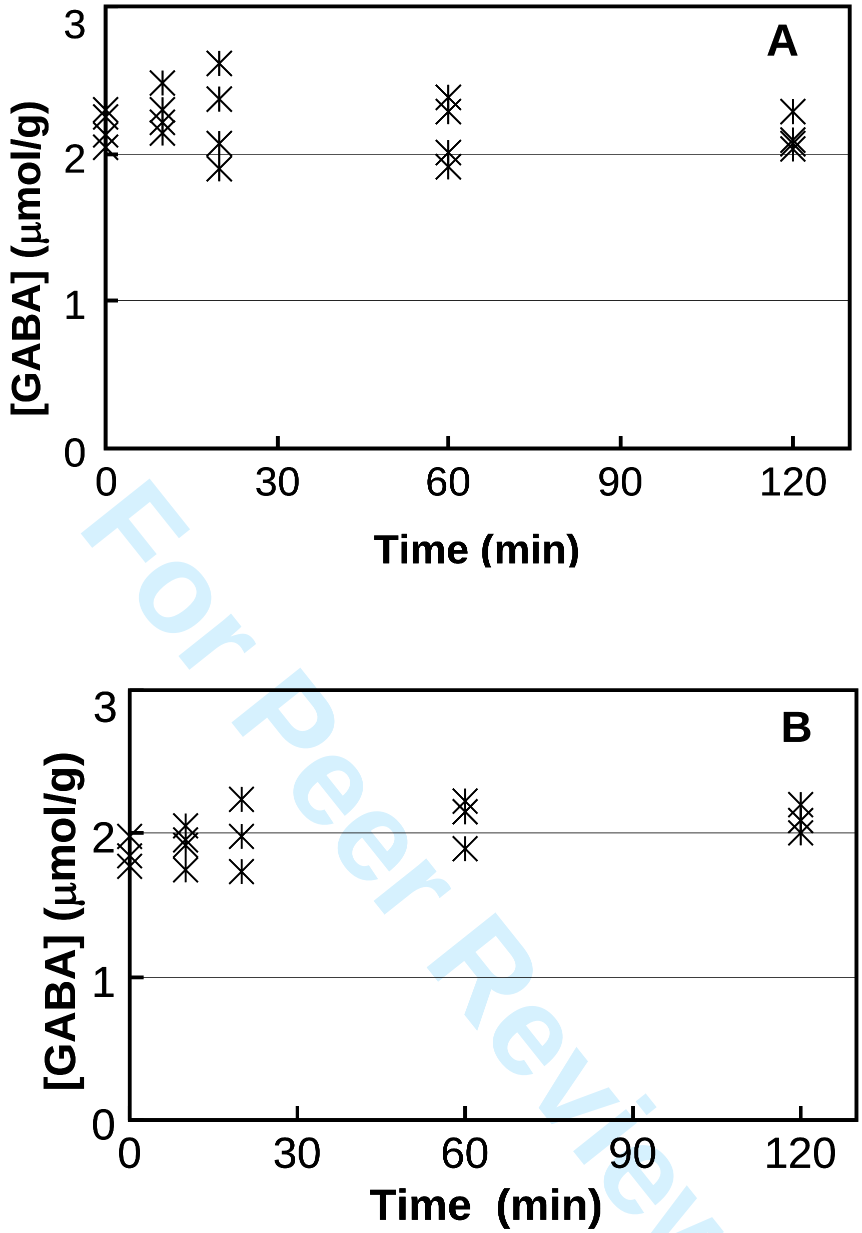

Fig. 1S. Time course of the concentration of GABA in striatum (A) Saline, (B) Nicotine.

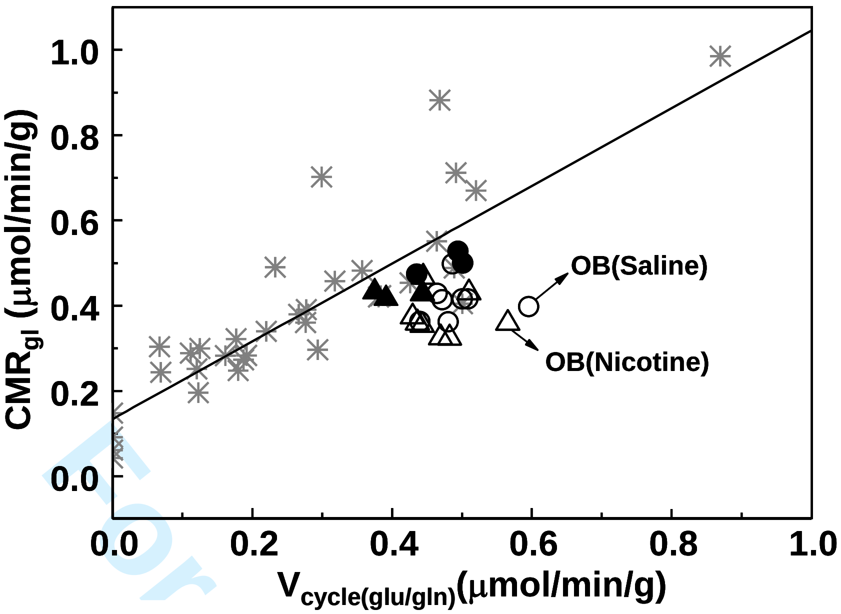

Fig. 2S. Correlation between the rate of glucose oxidation (CMR$_{gl(ox)}$) and glutamate neurotransmitter cycling (V$_{cycle}$). The plot combined the data from the present study (control group = ●; nicotine =▲) and the data from former studies (∗) (Patel et al. 2004, Sibson et al. 1998). The data from other regions were also added (saline = ○; nicotine = Δ). The line represents the regression analysis of both the wake and anaesthetized rat cortex, given by the equation $y = 0.912x + 0.134$, with a correlation coefficient of $r = 0.8672$.

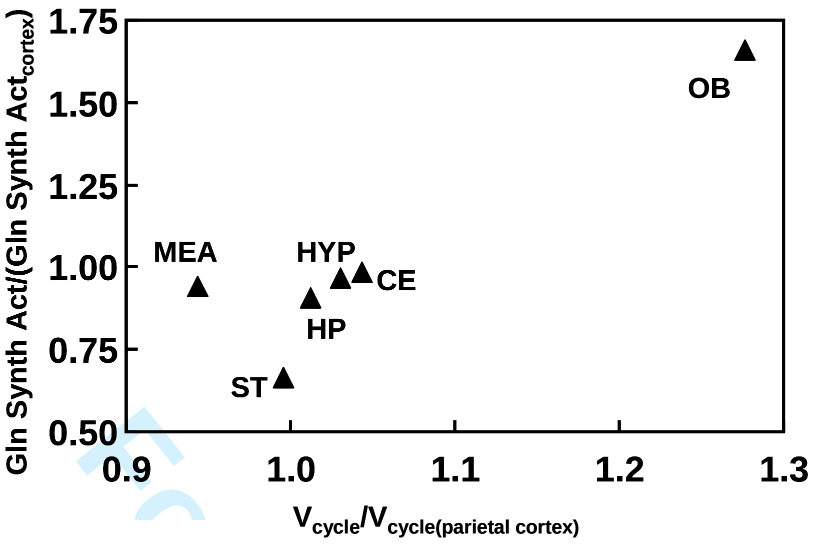

Fig. 3S: Relationship between glutamine synthetase activity in each region, taken as a ratio relative to the cortex, and the ratio of V_{cycle} in each region as a ratio relative to the value we measured for V_{cycle} in the parietal cortex. The regions for which glutamine synthetase data were available were the OB, ST, CE, HP, HYP, and MEA.

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