

1 **Abstract:** Postoperative abnormal neurobehavioral performance (PANP) is a common phenomenon
2 in the early postoperative period. The disturbed homeostatic status of metabolites in the brain after
3 anesthesia and surgery might make a significant contribution to PANP. The dynamic changes of
4 metabolites in different brain regions after anesthesia and surgery, as well as the potential association
5 with PANP is still not well understood. Here, a battery of behavioral tests was used to assess the
6 effects of laparotomy under isoflurane anesthesia in aged mice, and the metabolites in 12 different
7 sub-regions of the brain at different time points were investigated by proton nuclear magnetic
8 resonance ($^1\text{H-NMR}$) spectroscopic technique. The abnormal neurobehavioral performance
9 occurred at 6h and/or 9h, and recovered at 24h after anesthesia/surgery. Compared with the control
10 group, the altered metabolites of the model group at 6h were aspartate (Asp) and N-acetyl-aspartate
11 (NAA), and these differences were mainly displayed in the cortex; while significant changes at 9h
12 occurred predominantly in the cortex and hippocampus, and the corresponding metabolites were
13 Asp and glutamate (Glu), these changes returned to baseline level at 24h. The altered metabolic
14 changes could have occurred as a result of the acute PANP, and metabolites of Asp and Glu in the
15 cortex and hippocampus could provide preliminary evidence to our understanding of the PANP
16 process.

17

18 **Keywords:** Postoperative abnormal neurobehavioral performance; $^1\text{H-NMR}$; Metabolites; Aspartate;
19 Glutamate.

20

1 **Introduction**

2 Emerging evidence indicates that surgery/anesthesia could generate many complications
3 during the perioperative period, such as neurocognitive disorders, found especially in the elderly ¹,
4 ². Homeostasis of cerebral metabolism is fundamentally essential for neurobehavioral functioning.
5 The disturbance of metabolites in the brain after anesthesia and surgery might contribute
6 significantly to postoperative neurobehavioral disorders. Unfortunately, the dynamic changes of
7 metabolites in the aging brain during the early postoperative period, as well as the potential role of
8 these dynamic changes in the pathophysiology of postoperative abnormal neurobehavioral
9 performance (PANP) in the frail brain remains unknown. Previous reports suggest that several
10 metabolites might serve as biomarkers in neurobehavioral disorders, such as the glutamate (Glu),
11 N-acetyl-aspartate (NAA), creatine (Cr), gamma-aminobutyric acid (GABA), and aspartate (Asp)
12 in human beings and in rodents³⁻⁵. However, the association between different presentations of
13 PANP and the corresponding metabolites is still not well documented in the aging brain.

14 As an ionizing radiation-free technique, the *in vivo* magnetic resonance spectroscopy (¹H-MRS)
15 could provide tissue chemical information, including NAA, an indicator of viable neuronal tissue
16 density⁶; Cr, an essential molecule in energy homeostasis of the central nervous system (CNS)⁷;
17 choline (Cho), a marker that presents the rate of membrane turnover⁸; Glu, the main primary
18 excitatory neurotransmitter in the CNS; and GABA, the most prevalent inhibitory neurotransmitter
19 in the CNS ⁹. However, there are still several limitations of its application, such as lower spectral
20 resolution, limited metabolites, and quantification of the metabolites. The *in-vitro* ¹H-NMR ideally
21 resolves these problems by optimizing the homogeneity of the magnetic field and the extracted
22 sample without interference of the macromolecules and lipids. With much higher spectral resolution,

1 there are more metabolites identified using the *in-vitro* ¹H-NMR method ^[10]. Combined with
2 different quenching metabolism methods, it has been extensively applied in neuroscience studies ^{[5,}
3 ^{11, 12]}, and the metabolic mapping techniques have been extensively used to explore the variation of
4 metabolites in various brain disorders ^[13, 14]. Thus, it may present a promising method for analyzing
5 the dynamic metabolic changes in different brain regions following anesthesia and surgery, therefore,
6 shedding light on the basic profile of PANP.

7 The purpose of this study was to assess the effects of anesthesia/surgery on neurobehavioral
8 performance in aged mice during the early postoperative period and to explore the potential
9 metabolic mechanism *via* a ¹H-NMR based dynamic mapping method, which may provide a
10 dynamic map of metabolic information of aging brains following anesthesia/surgery and might be
11 beneficial to our understanding of the postoperative abnormal neurobehavioral performance in the
12 early postoperative period.

13

14 **Materials and Methods**

15 **Animals**

16 The animal experimental protocol was approved by the Animal Care and Use Committee of
17 Peking University (Beijing, China, Certification number: LA201413). Female mice C57Bl/6 (16
18 months, 20-25g) were purchased from the Experimental Animal Center of Hubei Provincial
19 Academy of Prevention. All animals were housed in plastic cages (four in each cage) and maintained
20 on a 12-h light/dark (lights at 7:00 am-7:00 pm) cycle, with food and water available *ad libitum*. In
21 order to accustom the animal to human interaction and minimize stress, the animals were handled
22 daily for a week before the experimental day, such as grasping the animal, mildly touching its

1 skin/hair, scratching the animals for about one minute.

2 **Anesthesia and surgery treatment**

3 Mice were randomly assigned into either the surgery plus anesthesia group (n=40, 10 animals
4 per time point, including 0h, 6h, 9h and 24h) or the control group (n=10). The experimental
5 procedure was according to previous reports with minor modifications ^[15, 16]. Specifically, the
6 animal was initially anesthetized with 1.4% isoflurane (in 100% oxygen) in a transparent chamber
7 (RWD Life Science, Shenzhen, China). Fifteen minutes after induction, the mouse was carried out
8 from the induction chamber, and a face mask was used to maintain the anesthesia with a 16-gauge
9 sensor monitoring the isoflurane concentration. Then a longitudinal midline incision was made from
10 the xiphoid to the pubic symphysis, cutting through the skin, abdominal muscles and peritoneum.
11 Then, the incision was sutured layer by layer with 5-0 Vicryl thread. At the end of the procedure,
12 EMLA cream (2.5% lidocaine and 2.5% procaine) was applied to the wound area every 8 hours to
13 minimize the pain and stress from the surgery. When the surgery was completed, the mouse was
14 returned to the anesthesia chamber for up to two hours to receive the rest of the isoflurane anesthesia
15 consisting of 1.4% isoflurane in 100% oxygen. The body temperature of the animal was maintained
16 with a heating pad during anesthesia/surgery. The mice in the control group were placed in their
17 home cage with 100% oxygen for two hours. To minimize the impact of circadian rhythms,
18 anesthesia/surgery began at 8 am every day.

19 **Behavior Test**

20 Buried food test, open field test and Y maze were employed to measure the abnormal
21 neurobehavioral performance in different periods after surgery. All animals received these three
22 behavioral tests before the surgery, and the results were set as the baseline for further behavioral

1 analysis. Then all mice in the control and treated groups received these three tests again at 6, 9, 24
2 hours after anesthesia/surgery. The protocol of these tests were based on previous studies with slight
3 modifications ^[15, 17]. For the animals in the treated groups at certain periods (6h, 9h and 24h), the
4 animals were euthanized using the microwave irradiation approach under isoflurane anesthesia just
5 after the behavioral tests at the same time points.

6 To help habituation, all mice were moved to the behavioral room one hour before the behavior
7 tests. Four mice from each group performed each day, which ensured that all behavior tests were
8 finished within 1 hour, to minimize the impact of circadian rhythms. To avoid the influence of the
9 odor in the equipment, 70% ethanol was used to clean all the equipment after every trial. All the
10 behavioral data were analyzed with the animal tracking system (Smart 3.0, RWD Life science Co.,
11 LTD, China). The details of the experimental steps are described in the supplementary material.

12 **Brain Sample Preparation for the NMR Study**

13 To minimize the impact of post-mortem changes of brain metabolites, the mouse was
14 euthanized using the microwave method which was fully described in our previous work ^[5]. After
15 euthanasia, the mouse brain was removed and dissected into 12 different regions: olfactory bulb
16 (OB), prefrontal cortex (PFC), parietal cortex (PC), occipital cortex (OC), temporal cortex (TC),
17 striatum (STR), hippocampus (HP), thalamus (THA), hypothalamus (HYP), midbrain (MID),
18 medulla-pons (MED-PONs), cerebellum (CE). Separation of the brain regions was according to the
19 Allen brain atlas and previous publications ^[18, 19], and the method used is also illustrated in the
20 supplemental material (Fig. S1). The tissues were immediately weighed and stored at -80 °C for
21 further processing.

22 The protocol for tissue extraction is the same as that mentioned in our previous study ^[5], which

1 is briefly described here. HCl/methanol (0.1M, 100 μ L) was added to the frozen tissues, and the
2 tissue was homogenized with Tissuelyser for 1.5 minutes at a frequency of 20 Hz (Tissuelyser II,
3 QIAGEN, German). Ice-cold 60% ethanol (800 μ L) was further added to tubes and the mixture
4 homogenized again, before being centrifuged at 14,000 g for 10 minutes. The supernatant was then
5 collected. The extraction steps were repeated twice with 800 μ L 60% ethanol to extract the left
6 metabolites in the sediment. All the supernatants were collected and desiccated with the centrifugal
7 drying apparatus (Thermo Scientific 2010, Germany) and freezing vacuum dryer (Thermo,
8 Germany). The dried product was preserved for further NMR studies.

9 The dried product was successively dissolved in 60 μ L D₂O (Containing the inner standard, 3-
10 (trimethylsilyl) propionic - 2, 2, 3, 3, d₄ acid sodium salt (120 mg/L, TSP, 269913-1G, Sigma-
11 Aldrich)) and 540 μ L phosphate buffer (pH=7.2). The solution was mixed in a high-speed vortex
12 and centrifuged at 14,000 g for 15 min, and the supernatant was withdrawn and transferred to an
13 NMR tube for the NMR study.

14 **NMR Spectrum Acquisition**

15 ¹H-NMR spectra were acquired as in previous studies [5, 20]. The extracted samples were
16 measured with a Bruker Avance III 600 MHz NMR spectrometer (298 K) equipped with an inverse
17 cryogenic probe (Bruker BioSpin, Germany). The ¹H-NMR spectra were acquired with a standard
18 WATERGATE pulse sequence [21]. The following acquisition parameters were set for every sample:
19 p1 (90° pulse): 8.35 μ s; NS (number of scans): 256; Spectral width: 20 ppm; Dummy scans: 8;
20 Number of FID points: 32 k.

21 **NMR Data Processing**

22 All ¹H-NMR spectra were processed and analyzed with TOPSPIN (Version 2.1, Bruker

1 BioSpin) and a home-made software *NMRSpec* [22]. Firstly, the phase correction and baseline
2 distortion were manually completed in TOPSPIN. Then the corrected spectra were imported into
3 *NMRSpec* for spectrum alignment, peaks extraction, spectra integration, and the integration of
4 chemical related peaks. This software has been widely used in several metabolomics studies [5, 23,
5 24].

6 The chemical shifts of major amino acids were distributed in the areas of 1.20–4.46 ppm, and
7 thus this gap was extracted for further analysis. First of all, areas of all peaks (area under the curve)
8 in this gap were automatically calculated for further statistical analysis [5]. To compensate for the
9 different concentration, every peak area was normalized to the sum of all the peak areas in this gap
10 of its own spectrum prior to the discriminant analysis [18, 20, 25].

11 Furthermore, the absolute concentrations ($\mu\text{mol/g}$ wet weight) of the identified metabolites
12 were calculated with the related peak areas in spectrum of the sample, information of the internal
13 standard chemical (TSP, such as concentration, proton number) and weight of specimen. The
14 calculation equation is shown as following:

$$15 \quad C_{met} = \frac{A_{met}/(R_{met} * N_H)}{A_{TSP}} * (C_{TSP} * V_{TSP}) * 9/W_t \quad (1)$$

16 Where A_{met} and A_{TSP} are the relative areas of the related peaks of the detected metabolites and
17 TSP, and R_{met} is a constant value for a certain metabolite which was calculated from the ratio between
18 the partial NMR signal of the standard metabolite between the selected regions (almost pure
19 chemical signal) in a real sample and the whole proton signal in the standard spectrum; N_H is the
20 number of protons of the metabolites within the certain area of A_{met} ; C_{TSP} and V_{TSP} is the
21 concentration and volume of TSP standard solution added in the NMR tube respectively; W_t is the
22 total weight of the wet specimen, and 9 is the number of protons in the TSP.

1 **Statistical Analysis**

2 In the behavioral test, a baseline parameter of every mouse was taken into consideration for
3 abnormal neurobehavioral performance. The relative values of behavior at every time point (6 h, 9
4 h or 24 h postoperatively) were presented as a percentage compared with its baseline value. The
5 repeated behavioral test of limited duration could influence the performance of mice, thus only the
6 control group and the 24h group, both of which received behavior tests 3 times (6 h, 9 h and 24 h),
7 were used to justify the difference in neurobehavioral performance. Values for behavior
8 performance were compared using the Wilcoxon Mann-Whitney U test.

9 To discriminate the different metabolic patterns between the control and anesthesia/surgery
10 groups, the orthogonal partial least squares discriminant analysis (OPLS-DA) was applied. There
11 were 12 different brain regions involved. For clarity, the metabolic spectra and statistical analysis
12 of the PFC region were presented as a typical example to show the efficiency of the OPLS-DA
13 method. To determine the significant differences in the corresponding metabolite levels in the whole
14 brain, a one-way analyses of variance (ANOVA), followed by a least significant difference (LSD)
15 post-hoc test were used to analyze the different concentrations of the metabolites in every brain
16 region. The criterion for statistical significance is a probability value of 0.05. All data are presented
17 as means \pm SEM.

18

19 **Results**

20 **Anesthesia/surgery increased the latency to find food in a time-associated manner**

21 To assess the effects of anesthesia and surgery on the natural habits of mice, the buried food
22 test was employed in the current study (Fig.1 (A1-A3)). Compared with the control group, the

1 latency to find food in the anesthesia/surgery group was longer both at 6 hours (114.0%±15.0%
2 *versus* 69.7%±12.8%, $Z = -2.098$, $p = 0.036$, Fig. 1A1) and 9 hours (171.0%±29.6% *versus*
3 81.2%±12.1%, $Z = -2.342$, $p = 0.019$, Fig. 1A2) after anesthesia/surgery. However, we found no
4 significant difference in the latency at 24 h after the operation when compared with controls (Fig.
5 1A3). Taken together, these data suggest that the abdominal surgery plus isoflurane anesthesia could
6 undermine the natural ability of mice to find food and this process was time-associated.

7 **Anesthesia/surgery decreased the time spent in the center of the open field box in a time-**
8 **associated manner**

9 The open field test was used to evaluate whether anesthesia/surgery affected the emotion of
10 mice (Fig.2). The anesthesia/surgery decreased the time spent in the center region at both 6 h
11 (39.0%±8.9% *versus* 122.0%±32.6%, $Z = -2.075$, $p = 0.038$, Fig. 2A1) and 9 h (21.1%±6.9% *versus*
12 139.0%±49.1%, $Z = -2.532$, $p = 0.011$, Fig. 2A2) points, but not at the 24 h point (Fig. 2A3), which
13 indicates that the anesthesia/surgery had an adverse effect on the emotion of mice in a time-
14 associated manner. Furthermore, other parameters (e.g. latency to the center and total travel distance)
15 were also analyzed. There was no significant difference between these two groups at any time point
16 (Fig. 2B1-C3), suggesting that anesthesia/surgery did not cause motor dysfunction in this study.
17 Taken together, the results of these behavior tests were consistent with our hypothesis that
18 anesthesia/surgery could cause neurobehavioral disorder in a time-associated manner in aged mice.

19

20 **Anesthesia/surgery decreased the exploration in the novel arm of the Y maze in a time-**
21 **associated manner**

22 The spontaneous Y maze test was introduced to evaluate spatial learning and memory in mice

1 (Fig. 3). Compared with the control group, anesthesia/surgery did shorten the duration in the novel
2 arm at 6 h ($81.0\% \pm 11.7\%$ versus $116.0\% \pm 10.0\%$, $Z = -2.203$, $p = 0.028$, Fig. 3A1) after the
3 intervention, but not at 9 h (Fig. 3A2) and 24 h (Fig. 3A3) points. Furthermore, the entries to the
4 novel arm in the anesthesia/surgery group was significantly less than that in the control group at both
5 6 h ($81.7\% \pm 4.1\%$ versus $105.0\% \pm 3.8\%$, $Z = -2.950$, $p = 0.003$, Fig. 3B1) and 9 h ($84.7\% \pm 7.3\%$ versus
6 $107.0\% \pm 6.4\%$, $Z = -2.061$, $p = 0.039$, Fig. 3B2) points, but not at 24 h point (Fig. 3B3). In addition,
7 the total entries to the three arms and total travel distance were also analyzed. We found no significant
8 difference at any time point (Supplemental material Fig. S2: A1-B3). These results demonstrate that
9 anesthesia/surgery could impair spatial learning and memory in mice in both a time-associated and
10 motor-independent manner.

11

12 **¹H-NMR based metabolic information for the aged brain at different postoperative time points**

13 To obtain the dynamic changes of the concentrations of metabolites that may be related to the
14 abnormal neurobehavioral performance and anesthesia/surgery states, four different time points (0
15 h, 6 h, 9 h and 24 h after anesthesia/surgery) were selected to analyze the metabolites' information
16 with the ¹H-NMR spectra. There were 12 different brain regions involved in the current study, and
17 an example region (PFC) from different time points are illustrated in Fig. 3. As shown in Fig. 4, the
18 average normalization spectra of the different groups were collected, and the basic metabolic
19 information exhibited, including the metabolite name and the related chemical shift. It can be seen
20 that group '0 H' has the minimum spectrum height of aspartate, glutamate; and the maximum
21 spectrum height of alanine. However, without statistical analysis information, it is difficult to
22 compare the significant differences among different groups, therefore, further analysis is necessary.

1

2 **Metabolic patterns in different brain regions after anesthesia/surgery**

3 The contents of metabolites in the control group were set as the baseline and variations of the
4 metabolites were investigated and identified. The significant changes of metabolites are listed in
5 Table 1. The level of metabolites in every region almost exhibited time-associated patterns
6 (maximum variation at 0 h, returned to normal levels at 24 h). Most of metabolites dramatically
7 decreased in most brain regions (except in the midbrain) after anesthesia/surgery; except for Ala
8 that increased in the 0 H group. The metabolites in most brain regions fully recovered to normal
9 levels (baseline) after 6 h except in the cortex regions. There were eight different brain regions
10 involved (FC, PC, OC, TC, STR, HP, THA and MID) after 9 h, and most metabolites returned to
11 baseline level after 24 h except for OB, FC, TC and CE.

12

13 **Discriminant analysis between the control and anesthesia/surgery treated groups**

14 The OPLS-DA method was used to select the dominant metabolite changes caused by
15 anesthesia/surgery and to visually discriminate the samples in different groups. There were 12
16 different brain regions analyzed. Among these brain regions, the metabolites in the PFC region
17 changed during the whole period. It was therefore, selected as an example of the brain regions to
18 illustrate the results of OPLS-DA (Fig. 5). The significant different metabolites corresponding to
19 anesthesia/surgery treatment were screened out with correlation coefficients of the OPLS-DA
20 method ($r > 0.4329$, $p < 0.05$, $F = 19$).

21 Compared with the control group, the anesthesia/surgery group showed multiple significant
22 changes at 0 h (Fig. 5A2). For instance, there was a decrease in glutamate and aspartate,

1 accompanied by an increase in alanine. There was also an observable increase in aspartate both at 6
2 h (Fig. 5B2) and 9 h (Fig. 5C2); but an increase in glutamate and a decrease in GABA were observed
3 only at 9 h (Fig. 5C2). In addition, there was a tendency for choline to increase and for glutamate to
4 decrease 24 h postoperatively (Fig. 5 D2).

5 Considering the vital function of the brain excitatory amino acid, the dynamic changes in
6 glutamate and aspartate were also further investigated (Fig. S3 and S4). Dynamic changes of Glu
7 and Asp in PFC are illustrated in Fig. 6A. Our results demonstrate that anesthesia/surgery treatment
8 sharply reduced the level of aspartate at 0 h (red line); there was a gradual reversal of the trend at 6
9 h (green line) and 9 h (blue line), but the level in aspartate returned closely to the baseline (control
10 group, pink line) after 24 h (yellow line). The other excitatory amino acid - glutamate presented a
11 similar variation tendency as the aspartate (Fig. 6B).

12

13 **The variation tendency in glutamate and aspartate in the whole brain after anesthesia/surgery**

14 To further assess the effects of anesthesia/surgery treatment on glutamate and aspartate in the
15 whole brain, their absolute concentrations were calculated and compared (Fig. 7 and Fig. 8). The
16 effects of anesthesia/surgery on aspartate were mainly reflected in the cortex (FC, PC, OC and TC)
17 at certain time points (0 h, 6 h and 9 h postoperatively) and their variation tendency of aspartate was
18 almost similar. Furthermore, the variation tendency of glutamate caused by anesthesia/surgery
19 mostly occurred in FC, TC, STR, HP and THA (Fig. 8) at certain time points (0 h and 9 h).

20 Collectively, these results suggest that anesthesia/surgery treatment could cause a
21 neurobehavioral disorder in a time-associated manner in aged mice. The anesthesia/surgery could
22 also alter some metabolites' dynamics (e.g. glutamate, GABA, alanine, aspartate, choline, NAA,

1 glutamine and glycine), and variations of some metabolites (e.g. glutamate and aspartate) were
2 consistent with the dynamic changes of the abnormal behavior performance in mice with
3 anesthesia/surgery challenge. The alteration of metabolites was also exhibited in a time-associated
4 manner.

5

6 **Correlation between neurobehavioral and NMR measurements**

7 In order to demonstrate the association between the metabolic changes and behavioral
8 abnormality in the current study, the correlations between the changed behavior and the significantly
9 altered metabolites (Glu and Asp) were analyzed. The region of PFC was selected due to the most
10 metabolic changes in this region. The results are shown in (Fig. 9). The concentrations of the Asp
11 and Glu were positively correlated with the latency to food, especially for ASP. However, they were
12 negatively correlated with the other behaviors, such as duration in center, duration in novel arm, and
13 entries into novel arm. Among these relationships, the concentrations of Asp were significantly
14 negatively correlated with the duration ($p=0.041$) and entries in novel arm ($p=0.028$).

15

16 **Discussion**

17 **Application of *in-vitro* nuclear magnetic resonance spectroscopy**

18 There were two magnetic resonance spectra methods that could be used to analyze the changes of
19 the metabolites in animal models – *in vivo* MRS, and *in vitro* NMR. Generally, the *in vivo* MRS
20 method could provide more accurate region-specific information due to its high spatial resolution,
21 ability to longitudinally monitor the changes of metabolites and reduce animal numbers in most
22 studies. Moreover, it has the direct potential for clinical translational value. The most fundamental

1 requirement for *in-vivo* ¹H-MRS test was to keep the animal immobile during the whole data
2 collection process. As we know, there are only two ways to keep the animal immobile, continuous
3 anesthesia or the use of a movement restricted apparatus. Given that the PNAP model in this study
4 was constructed by isoflurane plus surgery, which meant anesthesia could likely be an important
5 factor in facilitating behavior or metabolite changes, thus the *in-vivo* ¹H-MRS was not used in this
6 study. The *in-vitro* NMR also involved anesthesia, but the dose and duration of anesthesia were far
7 less than that of the *in-vivo* ¹H-MRS. In addition, the *in-vivo* MRS only tested one specific region
8 in every trial. To screen the regional metabolic patterns of PANP, the mouse brain was divided into
9 12 different regions for NMR measurement, which meant more confounded effects from the
10 continuous administration of anesthesia if the *in-vivo* ¹H-MRS was selected.

11 **Abnormal neurobehavioral performance in aged mice after anesthesia/surgery**

12 All the behavioral tests in this study were closely dependent on the movement ability of mice.
13 The present results showed that the locomotor activity was not significantly impaired at any time,
14 suggesting that the abnormal neurobehavioral performance at 6 h or 9 h was not related to the motor
15 function. There may be some other confounders to make a definite conclusion, such as the effect of
16 olfaction to the buried food test ^[26], however, each element of intact attention, unsubdued
17 consciousness, normal emotion and organized thinking was indispensable for mice to perform
18 normally.

19 Our results are not completely the same as those from a previous study^[15], in which there was
20 no significant difference in the latency to eat food at 6 h and the time spent in the center at 9 h. This
21 discrepancy is probably due to the difference in research subjects (16 month-old *versus* 4 month-
22 old mice), which is consistent with previous consensus that aging is an independent risk factor for

1 postoperative neurobehavioral disorders^[27].

2

3 **Brain regions affected after anesthesia/surgery**

4 Our results showed that the most severely affected regions are all in the four areas of the
5 cerebral cortex and the HP. These regions are the most indispensable regions in the brain and play
6 distinct and complementary roles in the process of normal neurobehavioral function (e.g. learning
7 and memory, emotion regulation) ^[28, 29]. The PANP may occur simultaneously when any of the FC,
8 TC and HP is directly impaired or the mutual complementary effect is abnormal ^[30-32].

9 Our results are also consistent with recent clinical imaging work. It was reported that several
10 regions are involved in the pathology of neurobehavioral dysfunction after anesthesia/surgery,
11 including the cortex, HP, THA and CE. These were obtained using diverse technologies, such as the
12 single photon emission computed tomography (SPECT) and the xenon-enhanced computed
13 tomography to investigate the cerebral blood flow changes ^[33, 34], the diffusion tensor imaging (DTI)
14 to analyze the neuronal connection ^[35], and the blood-oxygen-level-dependent (BOLD) signal to
15 measure functional connectivity ^[36]. Thus, cerebral regions of the cortex and HP could play key
16 roles in the pathology of neurobehavioral disorders after anesthesia/surgery.

17 **Metabolic variation after anesthesia/surgery**

18 Many metabolites in the whole brain fluctuated significantly following anesthesia/surgery,
19 including Myo, Asp, Glu, Gln, GABA, Tau, and Ala. The general tendency was that the
20 concentration of excitatory amino acids decreased (e.g. Asp and Glu) and the concentration of
21 inhibitory amino acids (e.g. GABA and Tau) increased, which indicates that anesthesia/surgery may
22 disturb the balance of excitatory and inhibitory function in the brain. Most of these metabolic

1 alterations had almost disappeared and the neurobehavioral performance returned to normal after
2 24 h.

3 The elevated tendency of Asp in the cortex at 6 h and 9 h accompanied with evident
4 neurobehavioral disorders in this study is supported by a previous study, in which the increment of
5 Asp was accompanied by impairment of neurobehavioral performance ^[4]. There are two isomers of
6 Asp, L-Asp and D-Asp. The latter can trigger glutamate transmission and is able to activate the N-
7 methyl-D-aspartate (NMDA) receptor ^[37]; while the former is a critical building block of proteins
8 and can be converted to the latter with the aid of D-Asp racemase ^[38]. There are published data
9 indicating that the application of exogenous D-Asp could not only alleviate the cognition
10 impairment induced by the stimulus of neuropathic pain ^[39], but also enhanced the long term
11 potential (LTP) and improved cognition in aged mice ^[40]. We, therefore, concluded that there may
12 be a positive correlation between the content of D-Asp and cognition. However, it is reported that
13 the D-Asp decreased and its content accounted for less than 1% of the total Asp in adults, while the
14 content of L-Asp increased relatively ^[41]. This suggests that the metabolic changes detected in our
15 study were mainly L-Asp, and there might have been more D-Asp due to conversion from the L
16 type. The possible explanations of the increased Asp and the decline in mice cognition are as follows:
17 (1) the effects of D-Asp on cognition might be associated with the individual age, which was
18 confirmed by a previous study ^[42]; (2) excess D-Asp generated the “D-Asp excitotoxicity” as the
19 surplus of excitatory neurotransmitter glutamate; (3) the increase of Asp was a result of
20 compensatory feedback from the decreased D-asp. Nevertheless, the exact content of D-Asp needs
21 to be determined by future studies.

22 Imbalance of Glu is strongly linked with both acute and chronic neurodegeneration, which

1 could lead to the phenomenon of excitotoxicity through disturbing the steady state of calcium, the
2 balance of energy, the normality of neuronal death pathways, and so forth ^[43]. In the current study,
3 the excess Glu at 9 h may possess excitotoxicity in the brain and impair the cognitive related cerebral
4 regions, such as FC and HP. This speculation is supported by an animal study, in which increment
5 of Glu was associated with cognitive dysfunction ^[4]. However, Kroll *et al* reported that reduced Glu
6 might also be related to the impairment of cognition in asthma patients ^[44]. The paradoxical evidence
7 might be due to the different species as well as the time-course observed. In the former study, an
8 acute animal model with rats was investigated and the observation time-course was less than 24
9 hours; while the latter study was a clinical observation for the possible association of Glu and
10 cognition decline in asthma patients, a common chronic disease of the respiratory system.

11

12 **Limitations and perspective**

13 This study aimed to explore the metabolic patterns of the PANP associated with surgical treatment;
14 and several potential metabolites were preliminarily screened out through the dynamic mapping
15 technique. Regrettably, we cannot elaborate the concrete cause (anesthesia or surgery) of PANP due
16 to the lack of an anesthesia alone group. A ¹H-MRS study demonstrated that isoflurane anesthesia
17 indeed altered the brain metabolites of mice immediately after the anesthesia^[45]. But whether (or
18 how) the anesthesia alone affected the PANP in aged brain needs to be explored in a further study.

19

20 **Conclusions**

21 By means of the ¹H-NMR method, this study explored the PANP after anesthesia/surgery from
22 a metabolic perspective. Both neurobehavioral performance and concentrations of metabolites

1 exhibit a parallel pattern in a time-associated manner. The most fluctuated metabolites in the various
2 cerebral regions are two critical excitatory amino acids, aspartate and glutamate. The current study
3 may provide a dynamic map of metabolite alterations associated with the neurobehavioral disorders
4 following anesthesia/surgery, which may be conducive to ultimately reveal the metabolic
5 mechanism of neurobehavioral disorders postoperatively.

6

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14

15 **Author Contributions**

16 T. Liu, J. Wang, H. Xiang, F. Xu and X. Guo designed the research; T. Liu, Z. Li, J. He, N. Yang,
17 and J. Wang conducted the experiment; T. Liu, X. Tian, D. Han, Y. Li, H. Liu, J. Wang, and H. Xiang
18 analyzed the data; T. Liu, J. Wang, H. Xiang, A. Manyande and X. Guo wrote the manuscript, A.
19 Manyande reviewed & proofread. All authors have reviewed and approved the final manuscript.

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1 **References:**

- 2 [1] Pinho C, Cruz S, Santos A, Abelha FJ. Postoperative delirium: age and low functional reserve as
3 independent risk factors. *Journal of Clinical Anesthesia* 2016, 33: 507-513.
- 4 [2] Pendlebury ST, Lovett NG, Smith SC, Dutta N, Bendon C, Lloyd-Lavery A, *et al.* Observational,
5 longitudinal study of delirium in consecutive unselected acute medical admissions: age-specific rates
6 and associated factors, mortality and re-admission. *BMJ Open* 2015, 5: e007808.
- 7 [3] Allaili N, Valabregue R, Auerbach EJ, Guillemot V, Yahia-Cherif L, Bardinet E, *et al.* Single-voxel H-1
8 spectroscopy in the human hippocampus at 3 T using the LASER sequence: characterization of
9 neurochemical profile and reproducibility. *Nmr in Biomedicine* 2015, 28: 1209-1217.
- 10 [4] Cui B, Wu MG, She XJ, Liu HT. Impulse noise exposure in rats causes cognitive deficits and changes
11 in hippocampal neurotransmitter signaling and tau phosphorylation. *Brain Research* 2012, 1427: 35-43.
- 12 [5] Liu TT, He ZG, Tian XB, Kamal GM, Li ZX, Liu ZY, *et al.* Specific patterns of spinal metabolites underlying
13 alpha-Me-5-HT-evoked pruritus compared with histamine and capsaicin assessed by proton nuclear
14 magnetic resonance spectroscopy. *Biochimica Et Biophysica Acta-Molecular Basis of Disease* 2017, 1863:
15 1222-1230.
- 16 [6] Meyerhoff DJ, MacKay S, Bachman L, Poole N, Dillon WP, Weiner MW, *et al.* Reduced brain N-
17 acetylaspartate suggests neuronal loss in cognitively impaired human immunodeficiency virus-
18 seropositive individuals: in vivo 1H magnetic resonance spectroscopic imaging. *Neurology* 1993, 43:
19 509-515.
- 20 [7] Andres RH, Ducray AD, Schlattner U, Wallimann T, Widmer HR. Functions and effects of creatine in
21 the central nervous system. *Brain Res Bull* 2008, 76: 329-343.
- 22 [8] Boulanger Y, Labelle M, Khat A. Role of phospholipase A(2) on the variations of the choline signal
23 intensity observed by 1H magnetic resonance spectroscopy in brain diseases. *Brain Res Brain Res Rev*
24 2000, 33: 380-389.
- 25 [9] Maddock RJ, Buonocore MH. MR spectroscopic studies of the brain in psychiatric disorders. *Curr Top*
26 *Behav Neurosci* 2012, 11: 199-251.
- 27 [10] Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain
28 metabolites. *Nmr in Biomedicine* 2000, 13: 129-153.
- 29 [11] Wang J, Du H, Jiang L, Ma X, de Graaf RA, Behar KL, *et al.* Oxidation of ethanol in the rat brain and
30 effects associated with chronic ethanol exposure. *Proc Natl Acad Sci U S A* 2013, 110: 14444-14449.
- 31 [12] Gonzalez-Riano C, Garcia A, Barbas C. Metabolomics studies in brain tissue: A review. *J Pharm*
32 *Biomed Anal* 2016, 130: 141-168.
- 33 [13] Clausen MR, Edelenbos M, Bertram HC. Mapping the Variation of the Carrot Metabolome Using H-
34 1 NMR Spectroscopy and Consensus PCA. *Journal of Agricultural and Food Chemistry* 2014, 62: 4392-
35 4398.
- 36 [14] Peng JN, Patil SM, Keire DA, Chen K. Chemical Structure and Composition of Major Glycans
37 Covalently Linked to Therapeutic Monoclonal Antibodies by Middle-Down Nuclear Magnetic Resonance.
38 *Analytical Chemistry* 2018, 90: 11016-11024.
- 39 [15] Peng M, Zhang C, Dong Y, Zhang Y, Nakazawa H, Kaneki M, *et al.* Battery of behavioral tests in mice
40 to study postoperative delirium. *Sci Rep* 2016, 6: 29874.
- 41 [16] Yang SM, Gu CP, Mandeville ET, Dong YL, Esposito E, Zhang YY, *et al.* Anesthesia and Surgery Impair
42 Blood-Brain Barrier and Cognitive Function in Mice. *Frontiers in Immunology* 2017, 8.
- 43 [17] Nathan BP, Yost J, Litherland MT, Struble RG, Switzer PV. Olfactory function in apoE knockout mice.

1 Behavioural Brain Research 2004, 150: 1-7.

2 [18] Wang J, Zeng HL, Du H, Liu Z, Cheng J, Liu T, *et al.* Evaluation of metabolites extraction strategies
3 for identifying different brain regions and their relationship with alcohol preference and gender
4 difference using NMR metabolomics. *Talanta* 2018, 179: 369-376.

5 [19] Wang J, Du H, Ma X, Pittman B, Castracane L, Li TK, *et al.* Metabolic products of [2-(13) C]ethanol
6 in the rat brain after chronic ethanol exposure. *J Neurochem* 2013, 127: 353-364.

7 [20] Du H, Fu J, Wang S, Liu H, Zeng Y, Yang J, *et al.* 1H-NMR metabolomics analysis of nutritional
8 components from two kinds of freshwater fish brain extracts. *RSC Advances* 2018, 8: 19470-19478.

9 [21] Liu ML, Mao XA, Ye CH, Huang H, Nicholson JK, Lindon JC. Improved WATERGATE pulse sequences
10 for solvent suppression in NMR spectroscopy. *Journal of Magnetic Resonance* 1998, 132: 125-129.

11 [22] Liu Y, Cheng J, Liu HL, Deng YH, Wang J, Xu FQ. NMRSpec: An integrated software package for
12 processing and analyzing one dimensional nuclear magnetic resonance spectra. *Chemometrics and*
13 *Intelligent Laboratory Systems* 2017, 162: 142-148.

14 [23] Kamal GM, Wang XH, Yuan B, Wang J, Sun P, Zhang X, *et al.* Compositional differences among
15 Chinese soy sauce types studied by C-13 NMR spectroscopy coupled with multivariate statistical analysis.
16 *Talanta* 2016, 158: 89-99.

17 [24] Kamal GM, Yuan B, Hussain AI, Wang J, Jiang B, Zhang X, *et al.* C-13-NMR-Based Metabolomic
18 Profiling of Typical Asian Soy Sauces. *Molecules* 2016, 21.

19 [25] Zhang LM, Wang LM, Hu YL, Liu ZG, Tian Y, Wu XC, *et al.* Selective metabolic effects of gold nanorods
20 on normal and cancer cells and their application in anticancer drug screening. *Biomaterials* 2013, 34:
21 7117-7126.

22 [26] Lehmkuhl AM, Dirr ER, Fleming SM. Olfactory assays for mouse models of neurodegenerative
23 disease. *J Vis Exp* 2014: e51804.

24 [27] Maldonado JR. Delirium pathophysiology: An updated hypothesis of the etiology of acute brain
25 failure. *Int J Geriatr Psychiatry* 2017.

26 [28] Frank LM, Brown EN, Wilson M. Trajectory encoding in the hippocampus and entorhinal cortex.
27 *Neuron* 2000, 27: 169-178.

28 [29] Bannerman DM, Rawlins JNP, McHugh SB, Deacon RMJ, Yee BK, Bast T, *et al.* Regional dissociations
29 within the hippocampus - memory and anxiety. *Neuroscience and Biobehavioral Reviews* 2004, 28: 273-
30 283.

31 [30] Chang RYK, Nouwens AS, Dodd PR, Etheridge N. The synaptic proteome in Alzheimer's disease.
32 *Alzheimers & Dementia* 2013, 9: 499-511.

33 [31] Kang MG, Byun K, Kim JH, Park NH, Heinsen H, Ravid R, *et al.* Proteogenomics of the human
34 hippocampus: The road ahead. *Biochimica Et Biophysica Acta-Proteins and Proteomics* 2015, 1854: 788-
35 797.

36 [32] Focking M, Lopez LM, English JA, Dicker P, Wolff A, Brindley E, *et al.* Proteomic and genomic
37 evidence implicates the postsynaptic density in schizophrenia. *Molecular Psychiatry* 2015, 20: 424-432.

38 [33] Fong TG, Bogardus ST, Daftary A, Auerbach E, Blumenfeld H, Modur S, *et al.* Cerebral perfusion
39 changes in older delirious patients using 99mTc HMPAO SPECT. *Journals of Gerontology Series a-*
40 *Biological Sciences and Medical Sciences* 2006, 61: 1294-1299.

41 [34] Yokota H, Ogawa S, Kurokawa A, Yamamoto Y. Regional cerebral blood flow in delirium patients.
42 *Psychiatry and Clinical Neurosciences* 2003, 57: 337-339.

43 [35] Cavallari M, Dai WY, Guttman CRG, Meier DS, Ngo LH, Hshieh TT, *et al.* Neural substrates of
44 vulnerability to postsurgical delirium as revealed by presurgical diffusion MRI. *Brain* 2016, 139: 1282-

1 1294.

2 [36] Choi SH, Lee H, Chung TS, Park KM, Jung YC, Kim SI, *et al.* Neural network functional connectivity
3 during and after an episode of delirium. *Am J Psychiatry* 2012, 169: 498-507.

4 [37] D'Aniello G, Tolino A, D'Aniello A, Errico F, Fisher GH, Di Fiore MM. The role of D-aspartic acid and
5 N-methyl-D-aspartic acid in the regulation of prolactin release. *Endocrinology* 2000, 141: 3862-3870.

6 [38] D'Aniello S, Somorjai I, Garcia-Fernandez J, Topo E, D'Aniello A. D-Aspartic acid is a novel
7 endogenous neurotransmitter. *Faseb Journal* 2011, 25: 1014-1027.

8 [39] Palazzo E, Luongo L, Guida F, Marabese I, Romano R, Iannotta M, *et al.* D-Aspartate drinking solution
9 alleviates pain and cognitive impairment in neuropathic mice. *Amino Acids* 2016, 48: 1553-1567.

10 [40] Errico F, Nistico R, Napolitano F, Mazzola C, Astone D, Pisapia T, *et al.* Increased D-aspartate brain
11 content rescues hippocampal age-related synaptic plasticity deterioration of mice. *Neurobiology of*
12 *Aging* 2011, 32: 2229-2243.

13 [41] Dunlop DS, Neidle A, Mchale D, Dunlop DM, Lajtha A. The Presence of Free D-Aspartic Acid in
14 Rodents and Man. *Biochemical and Biophysical Research Communications* 1986, 141: 27-32.

15 [42] Errico F, Nistico R, Napolitano F, Oliva AB, Romano R, Barbieri F, *et al.* Persistent increase of D-
16 aspartate in D-aspartate oxidase mutant mice induces a precocious hippocampal age-dependent
17 synaptic plasticity and spatial memory decay. *Neurobiology of Aging* 2011, 32: 2061-2074.

18 [43] Benarroch EE. Glutamate transporters Diversity, function, and involvement in neurologic disease.
19 *Neurology* 2010, 74: 259-264.

20 [44] Kroll JL, Steele AM, Pinkham AE, Choi C, Khan DA, Patel SV, *et al.* Hippocampal metabolites in
21 asthma and their implications for cognitive function. *Neuroimage Clin* 2018, 19: 213-221.

22 [45] Boretius S, Tammer R, Michaelis T, Brockmoller J, Frahm J. Halogenated volatile anesthetics alter
23 brain metabolism as revealed by proton magnetic resonance spectroscopy of mice in vivo. *Neuroimage*
24 2013, 69: 244-255.

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1 **Figure legends:**

2 **Fig. 1.** The impact of time-associated anesthesia/surgery (6 h, 9 h and 24 h after anesthesia/surgery)
3 on animal behaviors assessed by the Buried food test and the Y maze test. Data is represented
4 by means \pm SEM. *: $p < 0.05$, **: $p < 0.01$, Mann-Whitney u test.

5 **Fig. 2.** The impact of time-associated anesthesia/surgery (6 h, 9 h and 24 h after anesthesia/surgery)
6 on animal behaviors assessed by the Open field test. (A1-A3) Time spent in the center; (B1-B3)
7 Latency to the center; (C1-C3) Total travel distance. Data is represented by means \pm SEM. *: $p <$
8 0.05 , **: $p < 0.01$, Mann-Whitney u test.

9 **Fig. 3.** The impact of time-associated anesthesia/surgery (6 h, 9 h and 24 h after anesthesia/surgery)
10 on animal behaviors assessed by the Y maze test. (A1-A3) Duration in the novel arm; (B1-B3)
11 Entries in the novel arm. Data is represented by means \pm SEM. *: $p < 0.05$, **: $p < 0.01$, Mann-
12 Whitney u test.

13 **Fig. 4.** The normalized average $^1\text{H-NMR}$ spectra for the frontal cortex samples in the control and
14 model groups at different time points (0 h, 6 h, 9 h, and 24 h after the anesthesia/surgery). The
15 horizontal axis represents the chemical shift of $^1\text{H-NMR}$ spectrum. *Note: Lowercase, the carbon*
16 *position connected with the hydrogen signal; Lac, lactate; Myo, myo-inositol; Cre, creatine; Ala,*
17 *alanine; Glx, glutamine + glutamate; Gly, glycine; Asp, aspartate; EtoH, ethanol; Tau, taurine;*
18 *GABA, gamma-aminobutyric acid; NAA, N-acetyl aspartate.*

19 **Fig. 5.** OPLS-DA scores and Coefficient-coded loadings plot the models in the frontal cortex at
20 different time points. (A1-D1) OPLS-DA scores at 0h, 6h, 9h, and 24h after anesthesia/surgery;
21 (A2-D2) Coefficient-coded loadings plot the models at 0h, 6h, 9h, and 24h after the
22 anesthesia/surgery. *Note: $t[1]p$, first predictive component; $t[2]o$, Orthogonal principal*

1 component score; 1, lactate; 2, alanine; 3, glutamate; 4, aspartate; 5, taurine; 6, ethonal + myo-
2 inositol ; 7, alanine + glutamate + glutamine ; 8, creatine; 9, N-acetyl aspartate; 10, gamma-
3 aminobutyric acid; 11, choline.

4 **Fig. 6.** The normalized average $^1\text{H-NMR}$ spectra of selected metabolites in the frontal cortex at
5 different periods after anesthesia/surgery (means \pm SEM). The horizontal axis represents the
6 chemical shift of $^1\text{H-NMR}$ spectrum. (A) Aspartate; (B) Glutamate. *Note: Pink background,*
7 *control; Red background, 0h; Green background, 6h; Blue background, 9h; Yellow background,*
8 *24h.*

9 **Fig. 7.** Concentrations of aspartate in distinct brain regions at different time points after
10 anesthesia/surgery. Statistical significant differences between groups were assessed by one-way
11 ANOVA followed by LSD post-hoc multiple comparison test (* $p < 0.05$, # $p < 0.01$). *Note: FC,*
12 *Frontal Cortex; PC, Parietal Cortex; OC, Occipital Cortex; TC, Temporal Cortex; STR,*
13 *Striatum; HP, Hippocampus; THA, Thalamus; HYP, Hypothalamus; OB, Olfactory Bulb ; MID,*
14 *Midbrain; MED-PONS, Medulla-Pons; CE, Cerebellum.*

15 **Fig. 8.** Concentrations of glutamate in distinct brain regions at different periods after
16 anesthesia/surgery. Statistical significant differences among groups were assessed by one-way
17 ANOVA followed by LSD post-hoc multiple comparison test (* $p < 0.05$, # $p < 0.01$). *The full*
18 *names of the regions are the same as Fig. 6.*

19 **Fig. 9.** Correlations of aspartate and glutamate in PFC at different periods after anesthesia/surgery.
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Table.1. Metabolites changed in the whole brain classified by regions

	0H	6H	9H	24H
OB	Myo -14.3% ↑ (<i>P</i> =0.001)			
	Asp -22.3% ↓ (<i>P</i> =0.016)			
	Glu -39.6% ↓ (<i>P</i> <0.001)			Gln -20.6% ↓ (<i>P</i> =0.017)
	Ala -76.9% ↑ (<i>P</i> <0.001)			
FC	Asp -22.2% ↓ (<i>P</i> <0.001)			
	Glu -20.2% ↓ (<i>P</i> <0.001)	Asp -13.0% ↑ (<i>P</i> =0.006)	Asp -16.6% ↑ (<i>P</i> =0.001)	Cho -32.6% ↑ (<i>P</i> =0.024)
	Ala -89.9% ↑ (<i>P</i> <0.001)	NAA -6.29% ↑ (<i>P</i> =0.025)	Glu -6.39% ↑ (<i>P</i> =0.017)	Gln -25.6% ↓ (<i>P</i> =0.012)
	Lac -56% ↑ (<i>P</i> <0.001)			
PC	Tau -8.74% ↑ (<i>P</i> =0.004)			
	Asp -11.7 ↓ (<i>P</i> =0.035)			
	Glu -17.8% ↓ (<i>P</i> <0.001)	Asp -15.8% ↑ (<i>P</i> =0.001)	Asp -13.1% ↑ (<i>P</i> =0.010)	
	Ala -65.4 ↑ (<i>P</i> <0.001)			
	GABA -13.4 ↓ (<i>P</i> =0.002)			
OC	Cre -18.6% ↓ (<i>P</i> =0.005)			
	Asp -16.2% ↓ (<i>P</i> =0.013)			
	Glu -20.1 ↓ (<i>P</i> <0.001)	Asp -15.5% ↑ (<i>P</i> =0.006)	Asp -15.8% ↑ (<i>P</i> =0.007)	
	Ala -67.7 ↑ (<i>P</i> <0.001)			
TC	Asp -19.6% ↓ (<i>P</i> =0.001)	Cho -30.6% ↓ (<i>P</i> =0.020)		
	Glu -14.1% ↓ (<i>P</i> <0.001)	Asp -11.6% ↑ (<i>P</i> =0.018)	Glu -6.7% ↑ (<i>P</i> =0.027)	Glu -6.7% ↓ (<i>P</i> =0.021)
	Ala -76.6% ↑ (<i>P</i> <0.001)	NAA -8.7% ↑ (<i>P</i> =0.014)		
STR	Tau -7.3% ↑ (<i>P</i> =0.006)			
	Glu -22.0% ↓ (<i>P</i> <0.001)		Glu -8.0% ↑ (<i>P</i> =0.011)	
	Ala -89.0% ↑ (<i>P</i> <0.001)			
HP	Tau -8.8% ↑ (<i>P</i> =0.003)			
	Glu -18.2% ↓ (<i>P</i> <0.001)		Asp -15.8% ↑ (<i>P</i> =0.020)	
	Ala -70.6% ↑ (<i>P</i> <0.001)		Glu -6.1% ↑ (<i>P</i> =0.004)	
THA	Tau -12.0% ↑ (<i>P</i> =0.006)			
	Glu -23.9% ↓ (<i>P</i> <0.001)		Glu -8.2% ↑ (<i>P</i> =0.039)	
	Ala -63.7% ↑ (<i>P</i> <0.001)			
HYP	Glu -19.1% ↓ (<i>P</i> =0.001)			
	Ala -56.0% ↑ (<i>P</i> <0.001)			
	GABA -29.8% ↑ (<i>P</i> =0.009)			
MID	Ala -71.9% ↑ (<i>P</i> =0.001)		Cho -12.5% ↓ (<i>P</i> =0.027)	
MED	Asp -36.8% ↓ (<i>P</i> <0.001)			
	Ala -23.2% ↑ (<i>P</i> =0.032)			
CE	Gly -15.9% ↓ (<i>P</i> =0.004)			
	Asp -34.0% ↓ (<i>P</i> <0.001)			
	Ala -30.2% ↑ (<i>P</i> =0.004)	Asp -18.3% ↑ (<i>P</i> =0.023)		Gln -30.8% ↓ (<i>P</i> =0.015)
	Gln -45.9% ↓ (<i>P</i> =0.002)			

2 Note: Variation of metabolites in distinct brain regions at different periods after anesthesia/surgery.

3 Statistical significant differences among groups were assessed by one-way ANOVA followed by

4 LSD post-hoc multiple comparison test.