ANESTHESIOLOGY

Tau Contributes to Sevoflurane-induced Neurocognitive Impairment in Neonatal Mice

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ANESTHESIOLOGY 2020; 133:595–610

EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- Pathologic aggregation of the neuronal microtubule-associated protein Tau is a hallmark of Alzheimer's disease
- Sevoflurane anesthesia induces Tau phosphorylation and cognitive impairment in neonatal but not in adult mice, but the molecular mechanisms underlying these age-dependent effects have not been previously reported

What This Article Tells Us That Is New

- Neonatal mice have higher brain Tau levels and higher brain concentrations of Nuak1, an enzyme that phosphorylates Tau, when compared with adult counterparts
- Neonatal mice have decreased mitochondrial activity and lower brain ATP concentrations when compared with adult counterparts
- Pharmacologic inhibition of Tau phosphorylation or enhancement of mitochondrial function in neonatal mice protects against sevoflurane anesthesia–induced cognitive deficits
- These observations suggest that developmental stage–dependent differences in mitochondrial activity and Tau phosphorylation can render neonatal mice more vulnerable to the development of Tauopathy and cognitive impairment after sevoflurane anesthesia

ABSTRACT

Background: Sevoflurane anesthesia induces Tau phosphorylation and cognitive impairment in neonatal but not in adult mice. This study tested the hypothesis that differences in brain Tau amounts and in the activity of mitochondria–adenosine triphosphate (ATP)–Nuak1–Tau cascade between the neonatal and adult mice contribute to the age-dependent effects of sevoflurane on cognitive function.

Methods: 6- and 60-day-old mice of both sexes received anesthesia with 3% sevoflurane for 2 h daily for 3 days. Biochemical methods were used to measure amounts of Tau, phosphorylated Tau, Nuak1, ATP concentrations, and mitochondrial metabolism in the cerebral cortex and hippocampus. The Morris water maze test was used to evaluate cognitive function in the neonatal and adult mice.

Results: Under baseline conditions and compared with 60-day-old mice, 6-day-old mice had higher amounts of Tau (2.6 ± 0.4 [arbitrary units, mean ± SD] *vs.* 1.3 ± 0.2; $P < 0.001$), Tau oligomer (0.3 ± 0.1 *vs.* 0.1 ± 0.1; $P = 0.008$), and Nuak1 (0.9 ± 0.3 *vs.* 0.3 ± 0.1; $P = 0.025$) but lesser amounts of ATP (0.8 ± 0.1 *vs.* 1.5 ± 0.1; $P < 0.001$) and mitochondrial metabolism (74.8 ± 14.1 [pmol/min] *vs.* 169.6 ± 15.3; $P < 0.001$) in the cerebral cortex. Compared with baseline conditions, sevoflurane anesthesia induced Tau phosphorylation at its serine 202/threonine 205 residues (1.1 ± 0.4 *vs.* 0.2 ± 0.1; $P < 0.001$) in the 6-day-old mice but not in the 60-day-old mice (0.05 ± 0.04 *vs.* 0.03 ± 0.01; $P = 0.186$). The sevoflurane-induced Tau phosphorylation and cognitive impairment in the neonatal mice were both attenuated by the inhibition of Nuak1 and the treatment of vitamin K_2.

Conclusions: Higher brain Tau concentrations and lower brain mitochondrial metabolism in neonatal compared with adult mice contribute to developmental stage–dependent cognitive dysfunction after sevoflurane anesthesia.

(ANESTHESIOLOGY 2020; 133:595–610)

Young children who have had multiple exposures to anesthesia and surgery may experience a greater risk of developing neurocognitive disorders, although a single exposure to anesthesia and surgery may not be associated with such disorders.[1,2] A recent prospective study revealed that children with multiple exposures to anesthesia/surgery do not have significant reductions in their intelligence quotients but develop impairments in processing speeds and fine motor abilities.[3] However, other findings show that even multiple exposures of anesthesia and surgery do not cause cognitive dysfunction in children.[4,5]

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are available in both the HTML and PDF versions of this article. Links to the digital files are provided in the HTML text of this article on the Journal's Web site (www.anesthesiology.org). Yang Yu and Y. Yang contributed equally to this work.

Submitted for publication April 20, 2019. Accepted for publication June 9, 2020. Published online first on July 14, 2020. From the Department of Anesthesia, Tianjin Medical University General Hospital, Tianjin, China (Yang Yu, Y. Yang, Yonghao Yu); the Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts (Yang Yu, Y. Yang, H.T., F.H., L.L., M.L., Y.D., Y.Z., Z.X.); the Department of Anesthesia, Xinhua Hospital of Shanghai Jiaotong University, Shanghai, China (H.T.); the Center for Cancer Research, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts (M.B., W.H.); the Endocrine Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts (A.K.); the Department of Anesthesia, Second Affiliated Hospital of Nanchang University, Nanchang, China (F.H.); Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, China (L.L.), Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China (M.L.); and the Department of Anesthesiology, Columbia University Medical Center, New York, New York (G.Y.).

 Anesthesiology 2020; 133:595–610. DOI: 10.1097/ALN.0000000000003452

<zdoi;. DOI: 10.1097/ALN.0000000000003452>

In preclinical studies, anesthetics have been reported to induce cognitive impairment, apoptosis, synaptic deficiency, neuroinflammation, Tau phosphorylation, and other damage in young animals.[2] These neurotoxic and cognitive impairment effects may also be mediated through endocrine abnormalities,[6–11] histone acetylation,[12] and epigenetic mechanisms.[13] Our previous studies show that sevoflurane anesthesia induces Tau phosphorylation and cognitive impairment in 6-day-old neonatal mice but not 60-day-old adult mice.[14–17] However, the underlying mechanism of these age-dependent effects remains unknown.

Tau is a neuronal protein that assembles and stabilizes microtubules.[18] Tauopathy, the pathologic aggregation of Tau protein in neurofibrillary or gliofibrillary tangles in the brain, is a hallmark of Alzheimer's disease neuropathogenesis.[19] Nuak1 is an 5'-AMP-activated protein kinase–related kinase[20] that can phosphorylate Tau protein at serine 356, which decreases Tau degradation, leading to accumulation of total Tau.[21] However, the role of Tau in developmental anesthesia neurotoxicity and cognitive impairment in neonatal mice remains largely unknown. Specifically, whether Tau contributes to the difference in cognitive function between neonatal and adult mice after general anesthesia remains to be investigated.

The objective of the present study is to gain mechanistic insights into why sevoflurane anesthesia induces Tau phosphorylation and cognitive impairment in neonatal but not in adult mice. We hypothesized that differences in Tau amounts and Tau phosphorylation in brain tissues between neonatal and adult mice contribute to age-dependent differences in cognitive function after administration of sevoflurane anesthesia. Therefore, we compared the amounts of Tau and phosphorylated Tau in the cortex and hippocampus of neonatal and adult mice of both sexes. We also compared the amounts of Nuak1, as well as adenosine triphosphate (ATP) concentrations and mitochondrial metabolism, in the brain tissues of these mice. We used a specific Nuak1 inhibitor, HTH-01-015,[22] as well as vitamin K_2, a mitochondrial electron carrier energy enhancer[23] with neuroprotective effects,[23–25] to further determine the extent to which variations in brain mitochondria activity, ATP concentrations, and amounts of Nuak1 and Tau between neonatal and adult mice contribute to differences in cognitive function after sevoflurane anesthesia.

Materials and Methods

Mice, Anesthesia, and Treatment

The animal protocol was approved by the Standing Committee on Animals at Massachusetts General Hospital, Boston, Massachusetts (protocol 2006N000219). Efforts were made to minimize the number of animals used in the studies. The article was written according to Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines.

Adult mice (C57BL/6J) were purchased from the Jackson Laboratory (USA). Neonatal mice of both sexes at postnatal day 6 were obtained from our own breeding. We anesthetized mice of both sexes with 3% sevoflurane for 2 h at postnatal days 6, 7, and 8 based on our previous data showing that this treatment causes neuroinflammation, Tau phosphorylation, and cognitive impairment in neonatal mice.[14,15] Given that it is difficult to identify the sex of 6-day-old mice with certainty and that the objective of the present studies was not to determine sex-dependent effects, we did not allocate equal numbers of neonatal female or male mice in each group. Rather, neonatal mice of both sexes were used in the studies.

Importantly, during revision of this article, we were asked to include adult male mice in additional studies to determine whether age-related differences existed between the neonatal mice of both sexes and the adult female mice also could appear between the neonatal mice of both sexes and the adult male mice. Therefore, at the completion of the present studies, we used the mixture of female and male 6-day-old mice (6-day-old mice of both sexes), and either female or male 60-day-old mice to determine the underlying mechanism by which the sevoflurane anesthesia only induced cognitive impairment in neonatal mice of both sexes. We refer to these 6-day-old mice of both sexes as the "neonatal mice of both sexes" or "postnatal day 6" (female plus male) in the article and figures, respectively. Similarly, we called the 60-day-old mice "adult mice" or "postnatal day 60" (female, male, or female plus male) in the article and figures, respectively. These mice were randomly assigned to the following groups: (1) baseline; (2) baseline plus dimethyl sulfoxide (vehicle of HTH-01-015); (3) baseline plus HTH-01-015; (4) baseline plus corn oil (vehicle of vitamin K_2); (5) baseline plus vitamin K_2; (6) sevoflurane; (7) sevoflurane plus dimethyl sulfoxide; (8) sevoflurane plus HTH-01-015; (9) sevoflurane plus corn oil; or (10) sevoflurane plus vitamin K_2. Based on the results (*e.g.*, immunohistochemistry, enzyme-linked immunosorbent assay [ELISA] studies) and power analysis of the data (*e.g.*, Western blot, behavioral studies) from previous studies,[14] we included at least 10 mice in each group for behavioral studies; six mice in each group for Western blots, polymerase chain reaction, and ATP assays; four mice in each group for ELISA and mass spectrometry studies; and three mice in each group for immunostaining studies. Note that the additional experiments during the revision phases included adult male mice; thus we used different groups of neonatal mice of both sexes and adult male mice in these additional experiments. These different groups of neonatal mice of both sexes and male adult mice were also randomly assigned into the baseline and sevoflurane anesthesia groups. We used the same time of separation from mother as performed in our previous studies.[14,15]

The mice were anesthetized on postnatal days 6, 7, and 8 or on postnatal days 60, 61, and 62, as described in our

previous studies,[14] with 3% sevoflurane plus 60% oxygen for 2 h daily for 3 days. The mice in the baseline group only received 60% oxygen at an identical flow rate in similar chambers. We continuously monitored concentrations of sevoflurane and oxygen using a gas analyzer (Dash 4000; GE Healthcare, USA) during anesthesia administration. Anesthesia chamber temperature was monitored and controlled by a feedback-based system with a DC temperature control system (World Precision Instruments Inc., USA), which controls and automatically adjusts temperature to keep mouse rectal temperature at 37 ± 0.5°C) *via* a warming pad placed under this chamber. Anesthesia with 3% sevoflurane for 2 h did not cause significant changes in skin and core temperature (Supplemental Digital Content, fig. 1, A and B, http://links.lww.com/ALN/C429) or blood values of pH, pO_2, and partial pressure of carbon dioxide as compared with the baseline (Supplemental Digital Content, fig. 1, C–E, http://links.lww.com/ALN/C429).

In the intervention studies, we treated mice with HTH-01-015 (10 mg/kg, dissolved in dimethyl sulfoxide at 0.45 μg/μl; Tocris Bioscience, United Kingdom)[22] or vitamin K_2 (100 mg/kg, dissolved in corn oil at 4.5 μg/μl; Sigma–Aldrich, USA)[25] through intraperitoneal administration 30 min before each of the three treatments of sevoflurane anesthesia on postnatal day 6, 7, and 8 mice. The dosage of HTH-01-105 (10 mg/kg) was chosen based on our own observation that 10 mg/kg HTH-01-105 was able to decrease the amounts of Tau-PS356 in the brain tissues of mice. The mice in the vehicle treatment group received 0.1 ml of vehicle (15 μl of corn oil or dimethyl sulfoxide dissolved in 1 ml of saline).

Finally, we compared the Tau amounts between postnatal day 60 female mice and postnatal day 60 male mice and between postnatal day 6 mice of both sexes and postnatal day 60 female or male mice to assess whether there were sex-dependent changes in Tau amounts between the neonatal mice of both sexes and the adult mice.

Morris Water Maze

Morris water maze tests were performed as described in our previous studies.[14,15] Please see the Supplemental Digital Content (http://links.lww.com/ALN/C429).

Brain Tissue Harvest, Lysis, and Protein Quantification

The mice were decapitated at the end of sevoflurane anesthesia on postnatal day 8 or postnatal day 62, and cortex and hippocampus were harvested. Please see the Supplemental Digital Content (http://links.lww.com/ALN/C429).

Reverse Transcriptase–Polymerase Chain Reaction

RNA was harvested and isolated from mouse cortex and hippocampus tissues. Real-time reverse transcriptase–polymerase chain reaction was performed using the QuantiTect SYBR green real-time polymerase chain reaction kit (Qiagen, USA). Please see the Supplemental Digital Content (http://links.lww.com/ALN/C429).

Western Blot

Total Tau and Nuak1 amounts were detected with anti-Tau 46 antibody (catalog no. T9450, 55 kDa, 1:2,000, Sigma–Aldrich) and anti-Nuak1 antibody (catalog no. ab37641, 74 kDa, 1:1,000, Abcam, USA). Tau-PS356 antibody (catalog no. ab75603, 55 kDa, 1:1,000, Abcam) was used to determine amounts of Tau phosphorylated at serine 356. AT8 antibody (Tau-PS202/PT205, catalog no. MN1020, 55 kDa, 1:500, ThermoFisher Scientific, USA) was used to detect amounts of Tau phosphorylated at its serine 202 and threonine 205 residues. T22 antibody (catalog no. ABN454, 55 kDa, 1:1,000, Millipore, USA) was used to measure the amounts of Tau oligomers. Finally, antibody used to detect nontargeted protein β-actin (42 kDa, 1:5,000, Sigma, USA) served as a baseline for loading differences in total protein amount. Western blot quantification was performed as described by Xie *et al.*[26]

ELISA

We used the mouse total Tau immunoassay ELISA kit (catalog no. MBS724062, MyBiosource, USA) to determine the total Tau amounts in the cortex and hippocampus of mice at postnatal day 8 or 62. Please see the Supplemental Digital Content (http://links.lww.com/ALN/C429).

ATP Measurement

Concentration of ATP in the cortex of mice was determined with an ATP colorimetric/fluorometric assay kit per manufacturer's protocol (catalog no. ab83355, Abcam) and using methods described in our previous studies.[27]

Immunohistochemistry

The mice were anesthetized with sevoflurane briefly (3% sevoflurane for 5 min) and perfused transcardially with phosphate-buffered saline, followed by 4% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. Mouse brain tissues were removed, stored at 4°C in paraformaldehyde, and frozen. We used a Leica cryostat (USA) at −18°C to cut 10-μm frozen sections from mouse brain hemispheres, specifically from the prefrontal cortex, for immunostaining. The sections were mounted onto slides (ThermoFisher Scientific) and fixed in acetone for 2 h. Please see the Supplemental Digital Content (http://links.lww.com/ALN/C429).

Multiplexed Quantitative Mass Spectrometry–based Phosphoproteomics

Multiplexed quantitative mass spectrometry-based phosphoproteomics was done using tandem mass tag technology on an Orbitrap Fusion mass spectrometer (ThermoFisher Scientific) applying the SPS-MS3 method. Specifically,

phosphopeptides were labeled with tandem mass tags reagents as described previously.[28,29] Tandem mass tags-labeled phosphopeptides were identified using a MS2 spectra and quantified with the MultiNotch (simultaneous precursor selection) MS3 method[28,30] in a data-dependent mode. Precursor ion selection for MS3 spectra was done based on low-resolution MS2 spectral data using the three most intense fragment ions.[31] The peptides were quantified based on tandem mass tags reported ion intensities in the collected MS3 spectra, as reported previously.[28,29] Phosphorylation site localization was assigned using Ascore.[32] Phosphopeptide quantitative data were normalized in a two-step procedure. First, the average intensity of each species (protein or phosphopeptide) was calculated and normalized to the median of all average intensities. Second, to account for any mixing errors, intensity of each species was normalized from the ratio of the median intensity for a given tandem mass tags channel to the median of all species intensities. Please see the Supplemental Digital Content (http://links.lww.com/ALN/C429).

Mitochondrial Metabolism on Seahorse XFp Extracellular Flux Analyzer

Mitochondria were isolated from the hippocampus of postnatal day 6 and 60 mice of both sexes. Please see the Supplemental Digital Content (http://links.lww.com/ALN/C429).

Statistics

Based on results (*e.g.*, immunohistochemistry and ELISA) and power analysis (*e.g.*, Western blot and behavioral studies) of data obtained from our previous studies,[14] inclusion of 10 mice/group for behavioral studies; 6 mice/group for Western blot, polymerase chain reaction, ATP, and mitochondrial metabolism assays; 4 mice/group for ELISA and mass spectrometry studies; and 3 mice/group for immunostaining studies would provide statistically significant results. Specifically, a sample size of 10 mice/group provides more than 90% power to detect a 1.4-fold difference using a two-sided *t* test at the 0.05 α level, assuming SD of 70%. A sample size of 4 to 6 mice/group provides more than 80% power to detect a 0.5-fold difference using two-sided *t* test at the 0.05 α level, assuming SD of 20%.[14] These assumed SDs were higher than what we saw in our data; therefore these power calculations are conservative. We present the data from biochemistry studies and escape latency of Morris water maze as means ± SD; and platform-crossing numbers from the Morris water maze are presented as median and interquartile range. Notably, in experiments comparing neonatal and adult mice, we defined adult mice as the reference group and set values of variables in the reference group mice without anesthesia for comparison. In experiments to assess the effects of sevoflurane anesthesia, we defined nonanesthetized neonatal or adult mice as the baseline and set values of their variables for comparison. All data were quantified and expressed as arbitrary units (Western blot, ATP, and ELISA) or real numbers (number of positive cells, and mitochondrial metabolism) of the reference group or baseline group.

Interaction between time and group factors was determined by using two-way ANOVA with repeated measurements to analyze the difference in learning curves (based on escape latency) between mice in the baseline and sevoflurane anesthesia groups in the Morris water maze test. Student's *t* test with Bonferroni correction was used to compare the difference in escape latency between mice in the baseline and sevoflurane anesthesia groups during each day of the Morris water maze test. The Mann–Whitney test was used to determine the difference between the baseline and sevoflurane anesthesia groups in terms of platform-crossing number. There were no missing data for variables of the Morris water maze test (escape latency and platform-crossing number) during data analysis. Kruskal–Wallis test was used to determine the difference in multiple comparisons when the data did not pass a normality test (*e.g.*, Tau ELISA studies in fig. 1). Student's *t* test was used to determine the difference in two-group comparison when data passed a normality test. Two-way ANOVA and Student's *t* test with Bonferroni correction were used to determine the effect of age (postnatal day 6 *vs.* postnatal day 60), treatment (baseline *vs.* sevoflurane anesthesia), and the interaction of age and treatment in terms of total amounts of Tau, Nuak1, Tau-PS356, AT8 (Tau-PS202/PT205), and T22. One-way ANOVA and individual Student's *t* test with Bonferroni correction were used to determine differences among groups in terms of total Tau, Nuak1, Tau-PS356, AT8 (Tau-PS202/PT205), and T22 amounts. Student's *t* test was used to compare oxygen consumption rate between mice in the baseline and experimental groups. Finally, data for baseline brain amounts of Tau, Nuak1, ATP concentration, and mitochondrial metabolism from female and male adult mice were pooled together and compared with those obtained from neonatal mice of both sexes by Student's *t* test. $P < 0.05$ was considered statistically significant, and significance testing was two-tailed. Note that adjusted *P* values of the Bonferroni correction were calculated by dividing real *P* values with the size in experiments, and adjusted *P* values are reported. Statistical analysis was conducted using GraphPad Prism software (version 8.0) and SPSS statistic software (version 21.0).

Results

Neonatal Mice Have Higher Brain Amounts of Tau and Tau Oligomers than Adult Mice

To evaluate whether the age-dependent neurocognitive responses to sevoflurane could be related to age-specific differences in brain amounts of Tau and phosphorylated Tau between neonatal and adult mice, we first compared the amounts of these proteins between 6- and 60-day-old mice in baseline and after repeated exposures to sevoflurane (fig. 1a). Western blot analysis of protein expressions revealed that

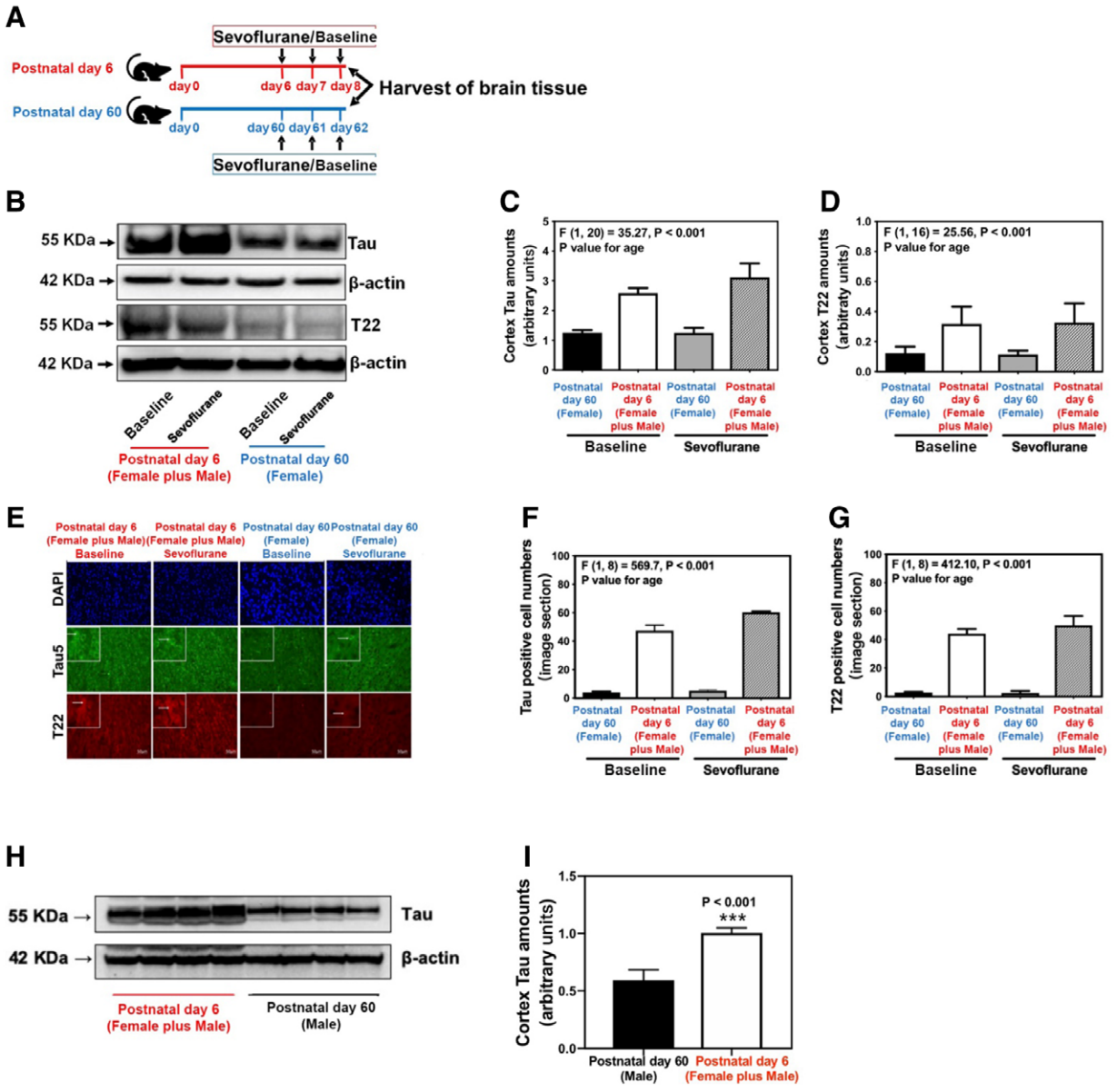

Fig. 1. Difference in brain Tau amount between neonatal and adult mice. (*A*) Experimental design. Cortex or hippocampus tissues were harvested at the end of the baseline or sevoflurane anesthesia in neonatal (postnatal days 6 to 8) and adult (postnatal days 60 to 62) mice. (*B*) Difference in brain amounts of Tau and oligomer Tau (T22) in the cortex of postnatal day 6 mice of both sexes (neonatal mice of both sexes) and postnatal day 60 female mice (female reference group mice) in baseline and sevoflurane anesthesia groups. (*C*) Summary of amounts of Tau (n = 6 mice/group). (*D*) Summary of amounts of oligomer Tau (T22; n = 6 mice/group). (*E*) Immunostaining of Tau- and T22-positive cells in the cortex of neonatal mice from of sexes and the female reference group mice in baseline and sevoflurane anesthesia groups. (*F*) Quantification of Tau-positive cells (n = 3 mice/group). (*G*) Quantification of T22-positive cells (n = 3 mice/group). (*H*) Difference in brain amounts of Tau and oligomer Tau (T22) in the cortex of postnatal day 6 mice from of sexes (neonatal mice of both sexes) and postnatal day 60 male mice (male reference group mice) in baseline. (*I*) Summary of amounts of Tau (n = 6 mice/group). The data are presented as means ± SD. All data are quantified and expressed as arbitrary units or real numbers compared with reference group mice (postnatal day 60) or baseline group (nonanesthetized mice). Two-way ANOVA was used for (*C*), (*D*), (*F*) and (*G*). Student's *t* test was used for (*I*). ***$P < 0.001$. DAPI, 4[prime],6[prime]-diamino-2-phenylindole.

6-day-old mice had greater amounts of Tau and Tau oligomers in the cortex than 60-day-old mice both in baseline (60-day-old female mice [reference group]: 1.3 ± 0.2 arbitrary unit *vs.* 6-day-old mice: 2.6 ± 0.4 arbitrary unit, $P < 0.001$) and after sevoflurane anesthesia (60-day-old female mice [reference group]: 1.2 ± 0.4 arbitrary unit *vs.* 6-day-old mice: 3.1 ± 1.2

arbitrary unit, $P = 0.004$; fig. 1, b and c). Tau oligomers were also comparably greater in the neonatal mice both in baseline (0.3 ± 0.1 [arbitrary unit] *vs.* 0.1 ± 0.1 [arbitrary unit], $P = 0.008$) and after sevoflurane anesthesia (0.3 ± 0.2 [arbitrary unit] *vs.* 0.1 ± 0.1 [arbitrary unit], $P = 0.009$; fig. 1, b and d). Immunohistochemical analysis (fig. 1, e–g) and ELISA measurements (Supplemental Digital Content, fig. 2, A and B, http://links.lww.com/ALN/C429) further confirmed the greater amounts of Tau and Tau oligomers in the cerebral cortex of neonatal mice compared with adult counterparts both in baseline and after sevoflurane exposures. Age-specific differences in Tau and Tau oligomer concentrations were also present in the hippocampus of the neonatal mice (Supplemental Digital Content, fig. 2, C–E, http://links.lww.com/ALN/C429).

In our original work, we compared Tau amounts in neonatal mice of both sexes to adult female mice. During the peer review process, we performed additional experiments to exclude potential sex-specific effects. We found that adult male mice, like their female counterpart, also had lower brain Tau amounts than the neonatal mice of both sexes (1.0 ± 0.1 arbitrary unit *vs.* 0.6 ± 0.1 arbitrary unit, $P < 0.001$; fig. 1, h and i), arguing against the potential sex-specific effect.

Repeated Exposures to Sevoflurane Induce Brain Tau Phosphorylation in Neonatal but Not in Adult Mice

Because Tau phosphorylation may impede the degradation of Tau, we next compared Tau phosphorylation between 6- and 60-day-old mice both in baseline and after sevoflurane exposure. Western blot analysis revealed that 6-day-old mice had higher amounts of phosphorylated Tau at serine 356 (Tau-PS356) and at serine 202 and threonine 205 (Tau-PS202/PT205) both in baseline and after repeated sevoflurane exposures when compared with 60-day-old female mice as a reference group (fig. 2, a–c). Repeated exposure to sevoflurane increased the amounts of Tau-PS202/PT205 in the cerebral cortex of the neonatal mice (1.1 ± 0.4 arbitrary unit *vs.* 0.2 ± 0.1 arbitrary unit, $P < 0.001$) but not of the adult mice (0.05 ± 0.04 *vs.* 0.03 ± 0.01, $P = 0.186$) as compared with the baseline nonanesthetized conditions (fig. 2, a–c). As in the cerebral cortex, Tau-PS365 and Tau-PS2020/PT205 were also higher in the hippocampus of neonatal mice compared with the female reference group (Supplemental Digital Content, fig. 2, C–E, http://links.lww.com/ALN/C429). In line with these observations, multiplexed quantitative mass spectrometry–based phosphoproteomics[29] showed more sites and higher amounts of pTau in the cortex of neonatal mice of both sexes compared with the female reference group mice (Supplemental Digital Content, fig. 3, A and B, http://links.lww.com/ALN/C429). In an additional sets of experiments, per the peer review request to exclude potential sex-specific effects, we compared and found no difference in Tau-PS356 and Tau-PS202/PT205 amounts between the cerebral cortex of adult male and female mice (Supplemental Digital Content, fig. 4, A–C, http://links.lww.com/ALN/C429).

Neonatal Mice Have Higher Brain Concentrations of Nuak1 than Adult Mice

Nuak1, an 5'-AMP-activated protein kinase–related kinase,[20] phosphorylates Tau protein at serine 356, which then decreases degradation of Tau and leads to accumulation of total Tau.[21] We therefore compared the amounts of Nuak1 in brain tissues between 6-day-old mice of both sexes and 60-day-old female mice (as a reference group) both in baseline nonanesthetized conditions and after repeated exposures to sevoflurane (fig. 3a). Neonatal mice of both sexes had lower amounts of phosphorylated Nuak1 in the cortex than the adult female (reference group) mice (fig. 3b). Moreover, neonatal mice of both sexes had higher amounts of Nuak1 in the cortex than the female reference group mice ($F = 41.43$, $P < 0.001$, two-way ANOVA and Student's *t* test with Bonferroni correction of three tests) in baseline group (0.9 ± 0.3 arbitrary unit *vs.* 0.3 ± 0.1 arbitrary unit, $P = 0.025$) or sevoflurane anesthesia group (0.9 ± 0.1 arbitrary unit *vs.* 0.3 ± 0.1 arbitrary unit, $P < 0.001$; fig. 3, c and d). Comparable findings were demonstrated in the hippocampus (Supplemental Digital Content, fig. 5, A and B, http://links.lww.com/ALN/C429). In contrast, no significant difference was found in mRNA expression of *Nuak1* in the cortex (fig. 3e) and hippocampus (Supplemental Digital Content, fig. 5C, http://links.lww.com/ALN/C429) between neonatal and adult mice. Per peer review request, we also compared Nuak1 concentrations in the cerebral cortex between neonatal mice of both sexes to adult male mice as a reference group and found comparable differences (60-day-old male mice: 0.5 ± 0.1 arbitrary unit *vs.* 6-day-old mice of both sexes: 0.8 ± 0.1 arbitrary unit, $P < 0.001$; fig. 3, f and g).

Because Nuak1 phosphorylation is an ATP-dependent process, we evaluated whether the lower level of Nuak1 phosphorylation observed in the neonatal mice is correlated with lower concentrations of ATP in brain tissues of neonatal *versus* adult mice. We found that the neonatal mice of both sexes exhibited lower baseline ATP concentrations in the cortex as compared with the female reference group mice (0.8 ± 0.1 arbitrary unit *vs.* 1.5 ± 0.1 arbitrary unit, $P < 0.001$; fig. 3h) or male reference group mice (fig. 3i). There were no significant differences in cortex Nuak1 amounts (Supplemental Digital Content, fig. 6, A and B, http://links.lww.com/ALN/C429) and ATP concentrations (Supplemental Digital Content, fig. 6C, http://links.lww.com/ALN/C429) between female and male postnatal day 60 mice either in the baseline nonanesthetized conditions or after sevoflurane exposure. Finally, sevoflurane anesthesia did not induce cognitive impairment in postnatal day 60 female (Supplemental Digital Content, fig. 6D, http://links.lww.com/ALN/C429) or male mice

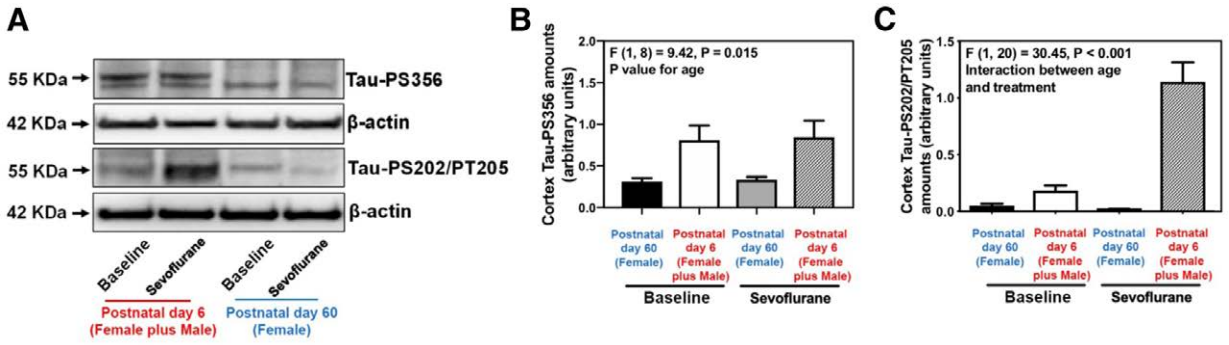

Fig. 2. Difference of brain phosphorylated Tau between neonatal and adult mice. (*A*) Difference in brain amounts of Tau–phosphorylated serine (PS) 356 and Tau–PS202/phosphorylated threonine (PT) 205 in the cortex of postnatal day 6 mice of both sexes (neonatal mice of both sexes) and postnatal day 60 female mice (female reference group mice) in baseline and sevoflurane anesthesia groups. (*B*, *C*) Summary of amounts of Tau–PS356 (*B*) and Tau–PS202/PT205 (*C*; n = 6 mice/group). All data are quantified and expressed as arbitrary units compared with reference group mice (postnatal day 60) or baseline group (nonanesthetized mice). Two-way ANOVA was used for (*B*) and (*C*).

(Supplemental Digital Content, fig. 6E, http://links.lww.com/ALN/C429).

Nuak1 Inhibitor HTH-01-015 Inhibits the Sevoflurane-induced Tau Phosphorylation and Cognitive Impairment in Neonatal Mice

To further test the hypothesis that lower ATP concentrations reduce Nuak1 phosphorylation in brain tissues of neonatal mice, thereby leading to higher Nuak1 and Tau amounts, we treated 6-day-old mice of both sexes with a specific Nuak1 inhibitor HTH-01-015[22] (fig. 4a). HTH-01-015 treatment did not affect brain ATP concentrations in the cerebral cortex of 6-day-old mice (fig. 4b). In contrast, Western blot analysis (fig. 4, c–e) and ELISA (fig. 4f) revealed that HTH-01-015 treatment did not alter total Tau amounts but decreased Tau oligomer amounts in the cortex and hippocampus (Supplemental Digital Content, fig. 7A-7E, http://links.lww.com/ALN/C429) of neonatal mice as compared with vehicle treatment.

In line with these observations, we also found that HTH-01-015 decreased the amounts of both Tau-PS356 and Tau-PS202/PT205 as compared with vehicle treatment in either the baseline or sevoflurane anesthesia (fig. 5, a–c). Comparable findings were obtained from hippocampal tissue (Supplemental Digital Content, fig. 7, C and D, http://links.lww.com/ALN/C429).

In 6-day-old mice, sevoflurane anesthesia induced cognitive impairment as measured *via* Morris water maze tests from P30–P36 on escape latency ($F = 5.40$, $P < 0.001$, two-way ANOVA) and platform-crossing number ($P = 0.004$, Mann–Whitney test; fig. 5, d and e) as compared with baseline nonanesthetized condition. However, in mice pretreated with HTH-10-105, sevoflurane anesthesia did not induce cognitive impairment, as evidenced by no significant interaction [$F = 0.71$, $P = 0.642$] between treatment and time on escape latency. Moreover, sevoflurane anesthesia did not decrease platform-crossing number as compared with baseline in mice pretreated with HTH-10-105 ($P = 0.541$, Mann–Whitney test; fig. 5, f and g).

Vitamin K_2 Mitigates the Sevoflurane Anesthesia–induced Tau Phosphorylation and Cognitive Impairment in Neonatal Mice

We next used vitamin K_2, a mitochondrial electron carrier and energy enhancer[23] with neuroprotective effects,[23–25] to determine whether increasing concentration of ATP and the resulting decrease in Nuak1-dependent Tau phosphorylation could protect neonatal mice from sevoflurane exposure-associated cognitive impairment (fig. 6a). In contrast to Nuak1 inhibitor HTH-01-015, vitamin K_2 increased brain ATP concentrations in neonatal mice of both sexes as compared with vehicle ($F = 101.40$, $P < 0.001$, one-way ANOVA and Student's *t* test with Bonferroni correction of five tests), which occurred in either the baseline (nonanesthetized, vitamin K_2: 1.1 ± 0.1 arbitrary unit *vs.* nonanesthetized, vehicle: 0.8 ± 0.1 arbitrary unit, $P < 0.001$) or sevoflurane anesthesia (sevoflurane, vitamin K_2: 1.1 ± 0.1 arbitrary unit *vs.* sevoflurane, vehicle: 0.7 ± 0.1 arbitrary unit, $P < 0.001$) groups (fig. 6b).

Vitamin K_2 decreased the amount of Nuak1 (fig. 6, c and d) and oligomer Tau (fig. 6, c and f), but not total Tau (fig. 6, c and e), in the cortex and hippocampus (Supplemental Digital Content, fig. 8, A and B, http://links.lww.com/ALN/C429) of neonatal mice of both sexes as compared with vehicle. ELISA showed that vitamin K_2 did not affect the Tau amounts in cortex (fig. 6g) and hippocampus (Supplemental Digital Content, fig. 8C, http://links.lww.com/ALN/C429) of 6-day-old mice.

Vitamin K_2 decreased amounts of Tau-PS356 and Tau-PS202/PT205 (fig. 7, a–c). Specifically, under baseline nonanesthetized conditions, we found that vitamin K_2

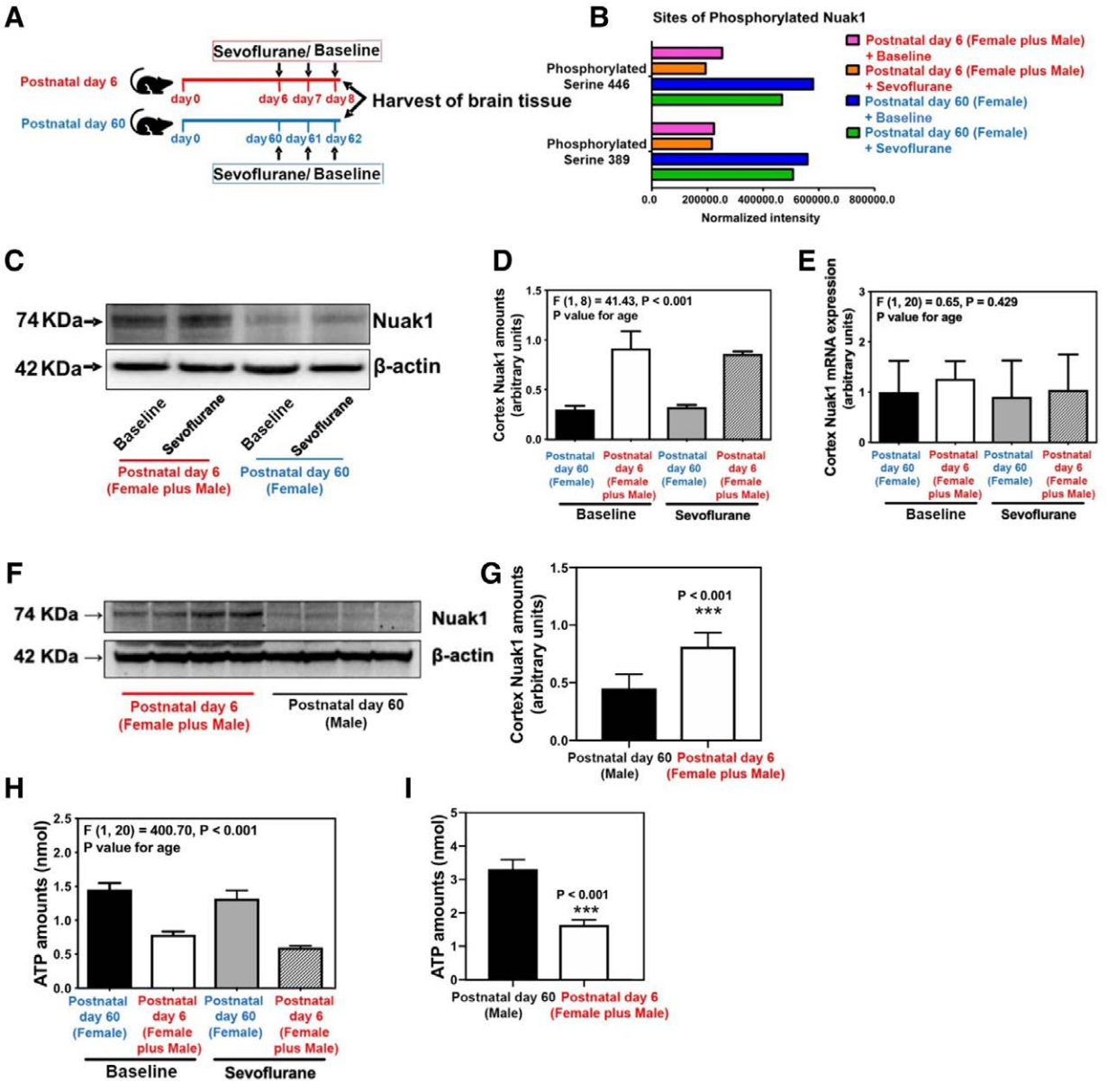

Fig. 3. Nuak1 and adenosine triphosphate (ATP) concentrations in brains of neonatal and adult mice. (*A*) Experimental design. Cortex and hippocampus were harvested at the end of the baseline or sevoflurane anesthesia in neonatal mice of both sexes and reference group mice. (*B*) Quantitative mass spectrometry data display amounts of phosphorylated Nuak1 in the cortex of neonatal mice of both sexes and female reference group mice in baseline and sevoflurane anesthesia groups. (*C*) Nuak1 protein amounts in the cortex of neonatal mice of both sexes and female reference group mice in baseline and sevoflurane anesthesia groups. (*D*) Summary of cortex Nuak1 protein amounts. (*E*) Difference in *Nuak1* mRNA expression in the cortex of neonatal mice of both sexes and female reference group mice in baseline and sevoflurane anesthesia groups. (*F*) Nuak1 protein amounts in the cortex of neonatal mice of both sexes and male reference group mice. (*G*) Summary of Nuak1 protein amounts in the cortex of neonatal mice of both sexes and male reference group mice. (*H*) ATP concentrations in the cortex of neonatal mice of both sexes and female reference group mice in baseline and sevoflurane anesthesia groups (n = 6 mice/group). (*I*) ATP concentrations in the cortex of neonatal mice of both sexes and male reference group mice (n = 6 mice/group). The data are presented as means ± SD. All data are quantified and expressed as arbitrary units or real numbers compared with reference group mice (postnatal day 60) or the baseline group (nonanesthetized mice). Two-way ANOVA was used for (*D*), (*E*), and (*H*). Student's *t* test was used for (*G*) and (*I*). ***$P < 0.001$.

decreased the amounts of Tau-PS202/PT205 as compared with vehicle, but the difference did not reach to statistical significance (nonanesthetized, vitamin K_2: 0.4 ± 0.2 arbitrary unit *vs.* nonanesthetized, vehicle: 0.7 ± 0.2 arbitrary unit, $P = 0.150$; fig. 7, a and c). In contrast, the sevoflurane anesthesia–induced increases in Tau-PS202/PT205 amounts

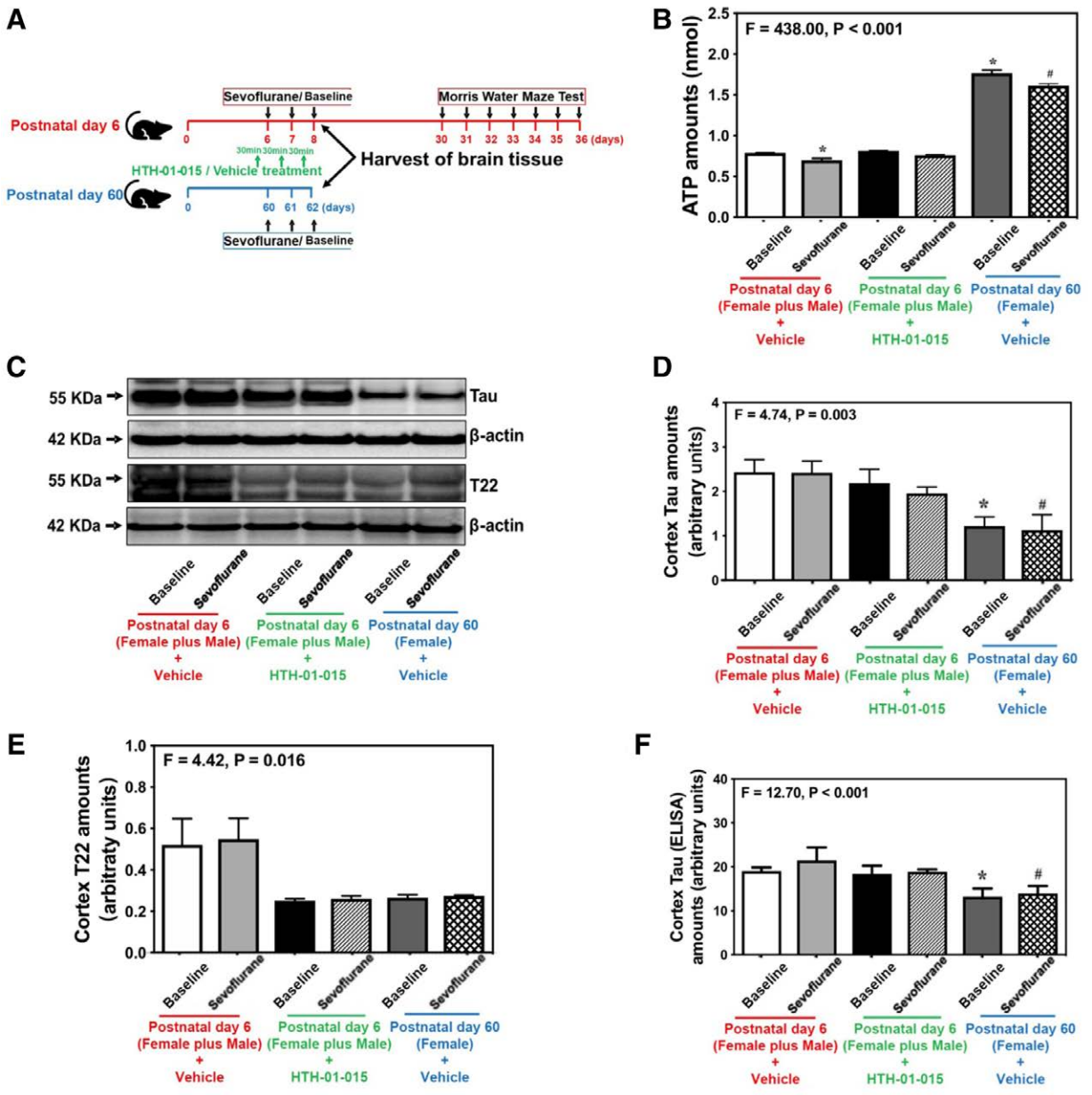

Fig. 4. Effects of Nuak1 inhibitor HTH-01-015 on Tau and oligomer Tau amounts in neonatal mice of both sexes. (*A*) Experimental design. The mice were treated with baseline or sevoflurane anesthesia with vehicle (dimethyl sulfoxide [DMSO]) or HTH-01-015. (*B*) ATP concentration in cortex of neonatal mice of both sexes and female reference mice in baseline and sevoflurane anesthesia groups (n = 6 mice/group). (*C*) Amounts of Tau and oligomer Tau (T22) in the cortex of neonatal mice of both sexes and female reference group mice. (*D*) Summary of Tau (n = 6 mice/group). (*E*) Summary of T22 (n = 6 mice/group). (*F*) Enzyme-linked immunosorbent assay (ELISA) of total Tau in cortex of neonatal mice of both sexes and female reference group mice (n = 6 mice/group). The data are presented as means ± SD. All data are quantified and expressed as arbitrary units or real number compared with baseline group (nonanesthetized mice). One-way ANOVA was used for (*B*), (*D*), (*E*), and (*F*). $^{*}P < 0.05$; $^{\#}P < 0.05$.

were significantly attenuated by vitamin K_2 (sevoflurane, vitamin K_2: 0.3 ± 0.2 arbitrary unit *vs.* sevoflurane, vehicle: 1.4 ± 0.4 arbitrary unit, $P = 0.011$) in cortex (fig. 7, a and c) and hippocampus (Supplemental Digital Content, fig. 8, A and B, http://links.lww.com/ALN/C429) of the neonatal mice.

The amounts of Tau-PS356 and Tau-PS202/PT205 in the cortex or hippocampus of neonatal mice of both sexes treated with vitamin K_2 were comparable to those in the cortex of female reference group mice treated with vehicle in baseline nonanesthetized conditions and after

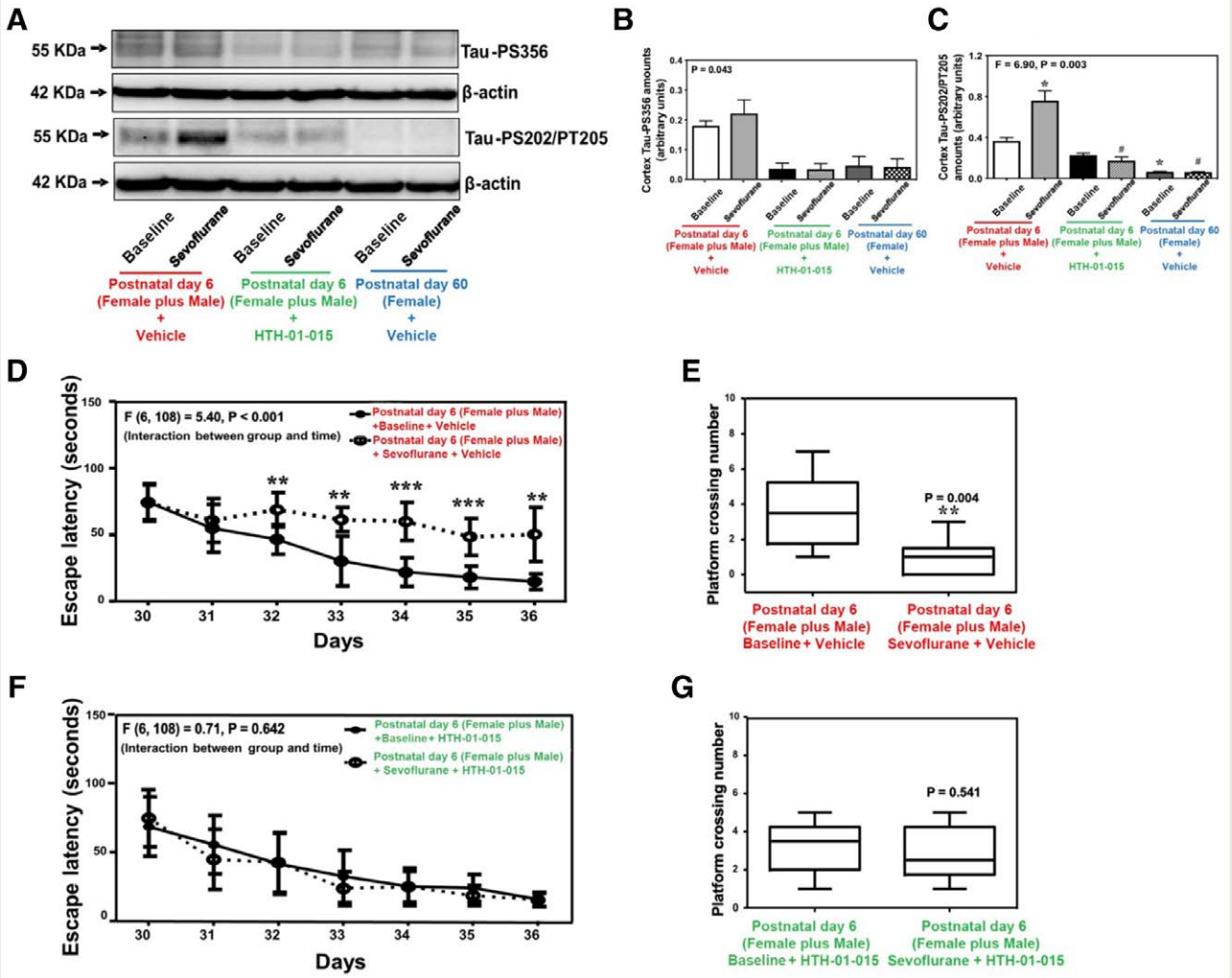

Fig. 5. Regulation of Tau phosphorylation and cognitive function by Nuak1 inhibitor HTH-01-015 in neonatal mice of both sexes. (*A*) Amounts of Tau–phosphorylated serine (PS) 356 and Tau–PS202/phosphorylated threonine (PT) 205 in the cortex of neonatal mice of both sexes and female reference group mice. (*B*) Summary of Tau-PS356 (n = 6 mice/group). (*C*) Summary of Tau-PS202/PT205 (n = 6 mice/group). (*D*, *E*) Escape latency (*D*) and platform-crossing number (*E*) of Morris water maze in neonatal mice for baseline and sevoflurane anesthesia groups after pretreatment with vehicle (n = 10 mice/group). (*F*, *G*) Escape latency (*F*) and platform-crossing number (*G*) of Morris water maze in neonatal mice for baseline and sevoflurane anesthesia groups after pretreatment with HTH-01-015 (n = 10 mice/group). The data are presented as means ± SD. All data are quantified and expressed as arbitrary units or real numbers compared with baseline group (nonanesthetized mice). One-way ANOVA was used for (*B*) and (*C*). Two-way ANOVA with repeated measurement was used for (*D*) and (*F*). Mann–Whitney test was used for (*E*) and (*G*). $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{\#}P < 0.05$.

sevoflurane exposure (fig. 7, a–c). Further, sevoflurane anesthesia induced cognitive impairment in Morris water maze tests in neonatal mice of both sexes pretreated with vehicle (fig. 7, d and e) but not in the neonatal mice pretreated with vitamin K_2 as compared with the baseline (fig. 7, *f* and *g*).

Brain Tissues of Neonatal and Adult Mice Have Different Mitochondrial Metabolism

Given the differences in brain ATP concentrations between neonatal mice of both sexes and reference group mice, we investigated the underlying mechanism by determining baseline mitochondrial metabolism in brain tissues of neonatal mice of both sexes and the reference group mice. Hippocampus mitochondrial metabolism in neonatal mice of both sexes was lower than those in female reference group mice, *e.g.*, basal respiration: 74.8 ± 14.1 pmol/min *vs.* 169.6 ± 15.3 pmol/min, $P < 0.001$ (fig. 8, a–d), and male reference group mice (fig. 8, e–h). There were no significant differences in hippocampus mitochondrial metabolism between female and male postnatal day 60 mice (Supplemental Digital Content, fig. 9, A–D, http://links.lww.com/ALN/C429).

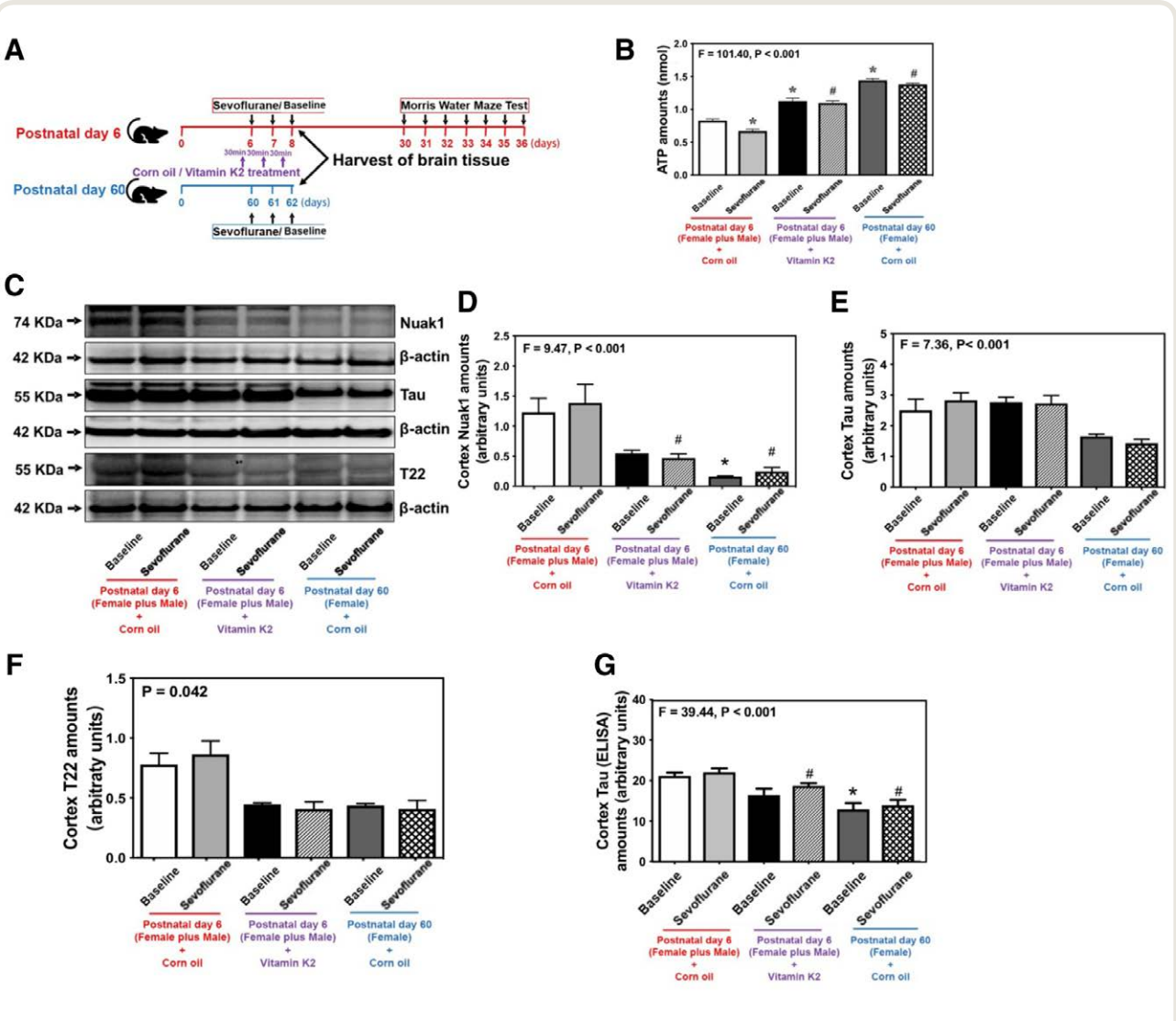

Fig. 6. Effects of vitamin K_2 on amounts of Tau and oligomer Tau in brain tissues of neonatal mice of both sexes. (*A*) Experimental design. (*B*) Adenosine triphosphate (ATP) concentration in cortex of neonatal mice of both sexes and female reference group mice after vehicle in sevoflurane anesthesia and baseline groups (n = 6 mice/group). (*C*) Amounts of Nuak1, Tau, and oligomer Tau (T22) in the cortex of neonatal mice of both sexes and female reference group mice. Summary of amounts of Nuak1 (*D*), Tau (*E*), and oligomer Tau (*F*; n = 6 mice/group). (*G*) Enzyme-linked immunosorbent assay (ELISA) of total Tau in the cortex of neonatal mice of both sexes and female reference group mice (n = 4 mice/group). The data are presented as means ± SD. All data are quantified and expressed as arbitrary units or real numbers compared with baseline group (nonanesthetized mice). One-way ANOVA was used for (*B*), (*D*), (*E*), and (*G*). Kruskal–Wallis test was used for (*F*). $^{*}P < 0.05$; $^{\#}P < 0.05$.

Discussion

This study provides new mechanistic insights into the age-dependent effects of sevoflurane on cognitive function. We found that 6-day-old neonatal mice have higher brain amounts of Tau and phosphorylated Tau proteins than adult mice both under baseline nonanesthetized conditions and after repeated exposure to sevoflurane. Moreover, neonatal mice had less baseline brain ATP concentrations and exhibited lower mitochondrial metabolism than adult mice. Finally, both Nuak1 inhibitor (HTH-01-015) and energy enhancer (vitamin K_2) attenuated sevoflurane-induced Tau phosphorylation and cognitive impairment in neonatal mice of both sexes. These data suggest the existence of a potential cascade of brain mitochondria–ATP–Nuak1–Tau and that higher baseline Tau amounts in brain tissues of neonatal mice contribute to age-dependent differences in sevoflurane anesthesia–induced Tau phosphorylation and cognitive function changes between neonatal and adult mice.

Previous studies demonstrate that anesthesia with one minimum alveolar concentration isoflurane for 4 h in

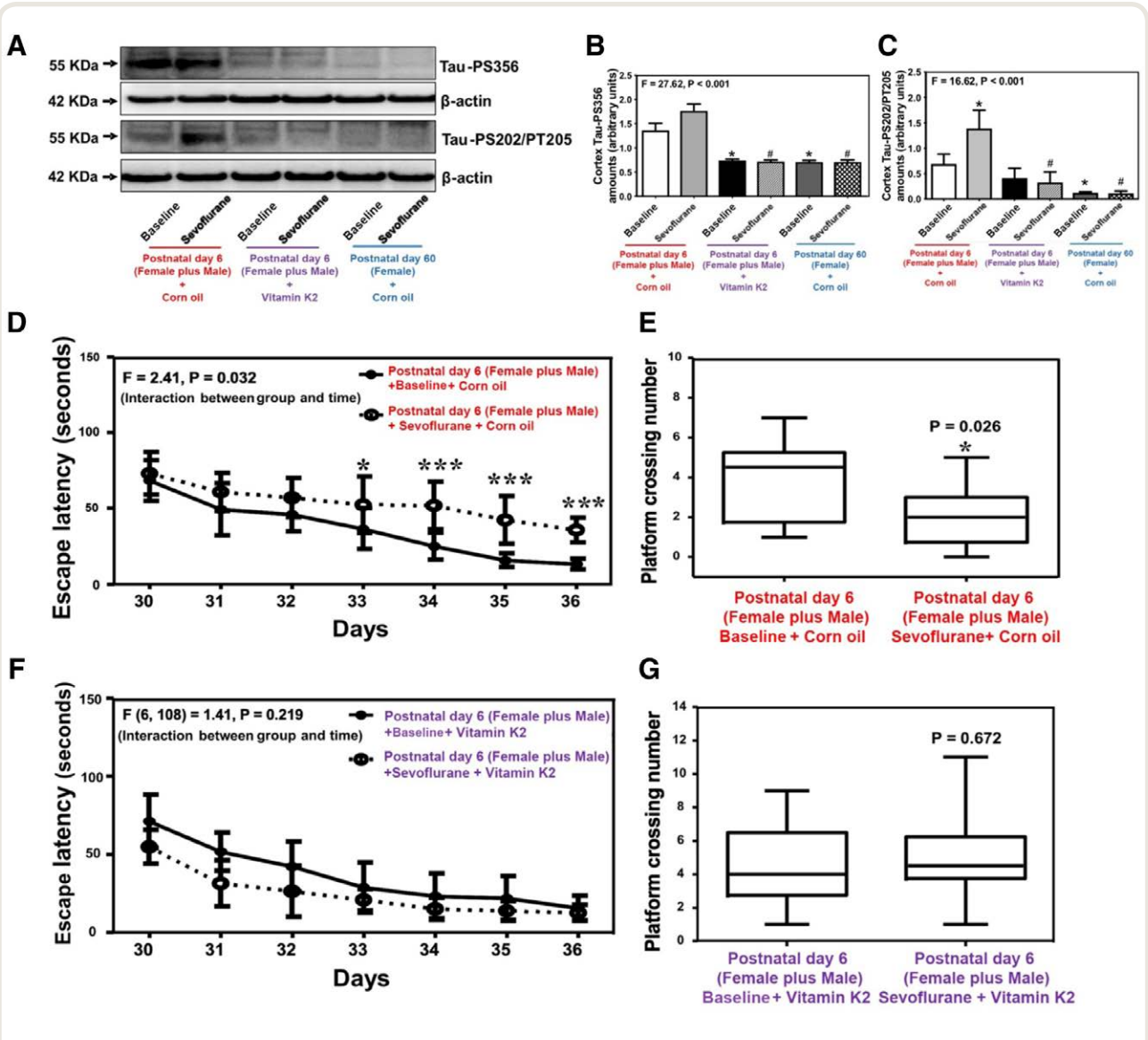

Fig. 7. Rescuing effects of vitamin K_2 on Tau phosphorylation and cognitive impairment in neonatal mice of both sexes. (*A*) Amounts of Tau phosphorylated serine (PS) 356 and Tau–PS202/phosphorylated threonine (PT) 205 in the cortex of neonatal mice of both sexes and female reference group mice. (*B*, *C*) Summary of amounts of Tau-PS356 (*B*) and Tau-PS202/PT205 (*C*; n = 6 mice/group). (*D*, *E*) Escape latency (*D*) and platform-crossing number (*E*) of Morris water maze test after vehicle treatment for sevoflurane anesthesia and baseline groups (n = 10 mice/group). (*F*, *G*) Escape latency (*F*) and platform-crossing number (*G*) of Morris water maze test after vitamin K_2 treatment for sevoflurane anesthesia and baseline groups (n = 10 mice/group). The data are presented as means ± SD. All data are quantified and expressed as arbitrary units or real numbers compared with baseline group (nonanesthetized mice). One-way ANOVA was used for (*B*) and (*C*). Two-way ANOVA with repeated measurement was used for (*D*) and (*F*). Mann–Whitney test was used for (*E*) and (*G*). $^{*}P < 0.05$; $^{\#}P < 0.05$.

postnatal day 7 rats induces neuronal death at 12 h after anesthesia in both males and females but only impairs recognition of objects and social memory deficits in male rats at postnatal day 38.[33] These results indicate there are potential sex difference in anesthesia neurotoxicity between male and female rats. Moreover, isoflurane anesthesia at postnatal day 4 induces greater neurotoxicity and neurobehavioral deficits than at postnatal day 7 in female but not male mice, suggesting sex- and age-dependent anesthesia neurotoxicity in developing brains.[34] This finding is consistent with other studies showing sex-dependent anesthesia neurotoxicity in developing brains.[13]

In the present study, however, we did not observe sex-dependent changes as evidenced by higher amounts of Tau, Nuak1, and Tau-PS356 but lower concentrations of ATP and mitochondrial metabolism in the brains of both neonatal mice of both sexes (postnatal day 6) of both sexes as compared with adult (postnatal day 60) female or male

mice. Moreover, there were no significant differences in the amounts of Tau, Nuak1, Tau-PS356, ATP concentration, mitochondrial metabolism, and cognitive function between female and male postnatal day 60 mice in the baseline and sevoflurane anesthesia groups. The reason for these differences between our results and previous studies is not known currently. However, previous studies used younger rodents (*e.g.*, 4 days old) and identified the sex of these rodents, whereas we used 6-day-old mice of both sexes. Nevertheless, our findings promote future research to determine whether there is sex-dependent developmental anesthesia neurotoxicity in mice.

Greater Nuak1 amounts were not due to more production but rather likely due to less protein metabolism, as evidenced by lower amounts of phosphorylated Nuak1 in brains of neonatal mice of both sexes as compared with adult mice. ATP plays an important role in protein phosphorylation.[35,36] In this regard, our results show that neonatal mice of both sexes had less baseline brain ATP and mitochondrial metabolism than adult mice. Nevertheless, greater Nuak1 amounts could also be due to other reasons, including lesser Nuak1 degradation. Future studies will seek to test this hypothesis by systematically comparing amounts of all Nuak1 metabolites in brain tissues between neonatal and adult mice by using mass spectrometry for screening and Western blot for confirmation.

The Nuak1 inhibitor HTH-01-015 and vitamin K_2 decreased the higher amounts of Tau-PS356, T22, and pTau (baseline and sevoflurane-induced) in the brains of neonatal mice of both sexes as well as sevoflurane-induced cognitive impairment. These data further suggest that differences in brain Nuak1 and energy amounts between neonatal and adult mice contribute to differences in brain Tau phosphorylation and cognitive function after administration of sevoflurane anesthesia. Notably, there were comparable amounts of brain Tau-PS356 and T22 between neonatal mice of both sexes treated with HTH-01-015 or vitamin K_2 and adult mice treated with vehicle. These findings suggest that targeting Nuak1 and energy may reduce the difference between neonatal and adult mice in relation to vulnerability to anesthesia-induced cognitive impairment in neonatal mice of both sexes.

HTH-01-015 did not increase brain ATP concentrations in neonatal mice of both sexes compared with vehicle, although the mitochondrial electron carrier and energy enhancer vitamin K_2 increased brain ATP concentrations in neonatal mice of both sexes. These data suggest that changes in ATP concentrations occur before alterations of Nuak1 amounts.

Moreover, vitamin K_2 also attenuated higher amounts of Tau-PS356, T22, pTau, and Tau- and T22-positive cells in the brains of neonatal mice of both sexes compared with vehicle. Further, vitamin K_2 mitigated sevoflurane anesthesia–induced brain Tau phosphorylation and cognitive impairment in neonatal mice of both sexes. These data further suggest that deprivation of brain energy in neonatal mice of both sexes is one of the underlying mechanisms by which neonatal mice of both sexes become more vulnerable to Tau phosphorylation and cognitive impairment after administration of sevoflurane anesthesia.

Interestingly, neither HTH-01-015 nor vitamin K_2 decreased total Tau amounts in the brains of postnatal day 6 mice. This could be attributed to the fact that the half-life of Tau is 9.7 days[37] and that we only harvested brain tissues 3 days after the first treatment of HTH-01-015 or vitamin K_2. Neonatal mice of both sexes may have lower concentrations of ATP and mitochondrial metabolism as compared with adult mice because of less glucose metabolism,[38] but this still remains to be determined in future studies.

Using mice overexpressing the human Tau transgene (hTau mice), Polydoro *et al.*[39] reported that 12-month-old hTau mice have higher incidences of Tauopathy, impaired synaptic transmission, and neurobehavioral deficits than 4-month-old hTau mice. Consistently, our data show age-dependent Tau phosphorylation and cognitive impairment after administration of sevoflurane anesthesia. However, the current studies further illustrate that such age-dependent changes in Tau phosphorylation and cognitive function could be due to the age-dependent cascade of mitochondria–ATP–Nuak1–Tau (Supplemental Digital Content, fig. 10, http://links.lww.com/ALN/C429), with neonatal mice of both sexes more vulnerable to development of Tau phosphorylation and cognitive impairment. Future studies are needed to further test this hypothesis using hTau mice.

Despite the aforementioned strengths, our studies also have several limitations. First, we did not use Western blot to confirm other Tau phosphorylation sites screened by mass spectrometry. However, the purpose of the current studies was to assess whether differences in amounts of brain Tau, Nuak1, ATP concentration, and mitochondrial metabolism between neonatal and adult mice contributed to differences of sevoflurane-induced Tau phosphorylation and cognitive impairment. We chose Tau-PS202/Tau-PT205 as representative of pTau for this purpose. Second, we did not identify male and female neonatal mice because of difficulties identifying sex in neonatal mice. However, our objective was to reveal the underlying mechanism by which neonatal mice were more vulnerable to development of Tau phosphorylation and cognitive impairment after sevoflurane anesthesia. Looking forward, we will use the established system to assess potential sex differences in Tau phosphorylation and cognitive function in neonatal mice. Third, we determined age-dependent changes in Tau metabolism, its upstream mechanism and downstream consequences in the whole cortex and hippocampus rather than in subregions of the hippocampus and cortex, so it is unknown whether age-dependent anesthesia neurotoxicity is dependent on specific brain regions. However, the objective of the present study was to compare overall baseline amounts of Tau, ATP, and mitochondrial metabolism, among others, in brain tissues of

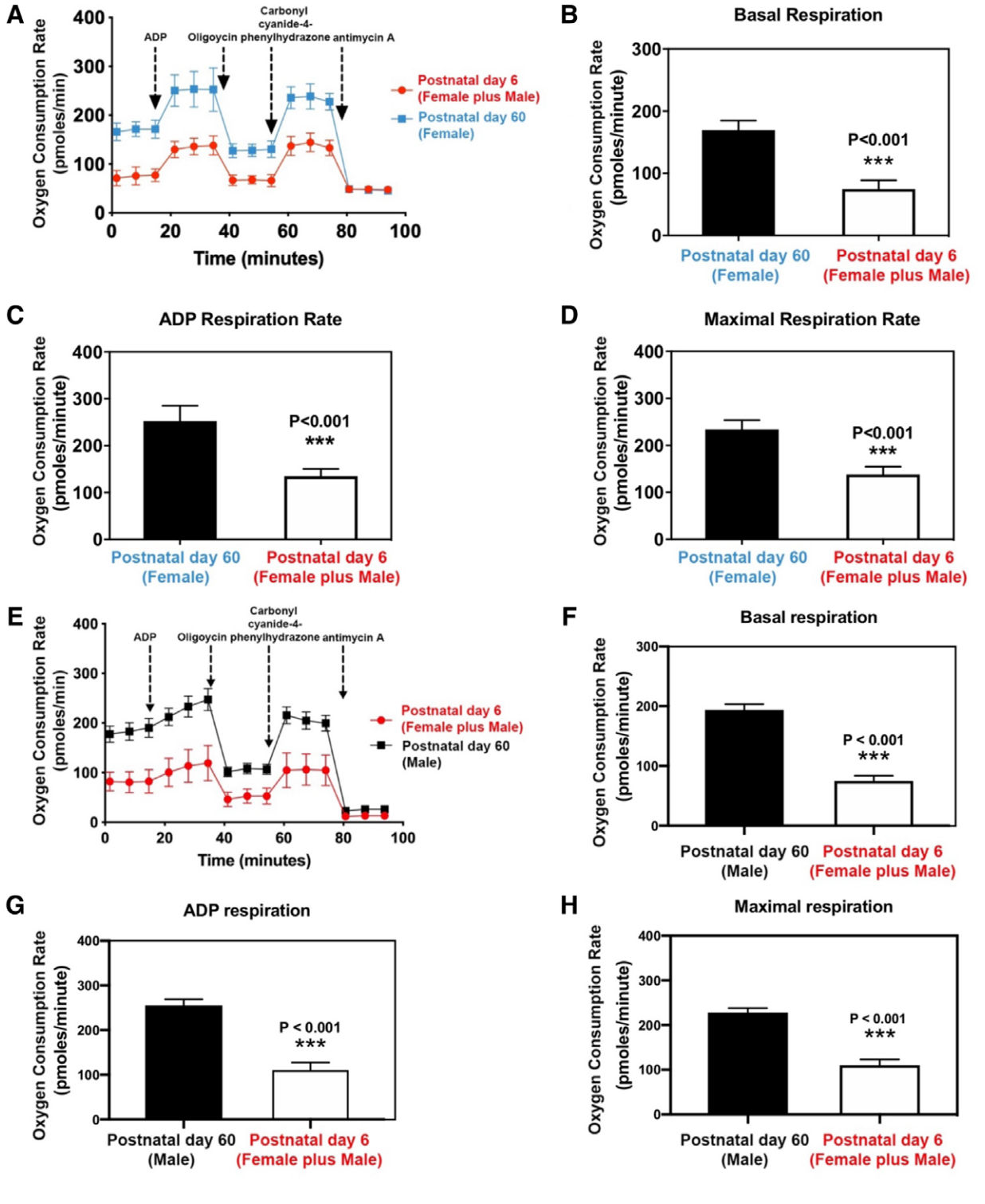

Fig. 8. Brain mitochondrial metabolism in neonatal and adult mice. (*A–D*) Overall lower oxygen consumption rate (*A*), basal respiration (*B*), adenosine diphosphate (ADP) respiration (*C*), and maximal respiration (*D*) in the hippocampus of neonatal mice of both sexes than female reference group mice (n = 6, Student's *t* test). (*E–H*) Overall lower oxygen consumption rate (*E*), basal respiration (*F*), ADP respiration (*G*), and maximal respiration (*H*) in the hippocampus of neonatal mice of both sexes than male reference group mice (n = 6, Student's *t* test). The data are presented as means ± SD. All data are quantified and expressed as real numbers compared with reference group mice (adult mice). Student's *t* test was used. ***$P < 0.001$.

neonatal and adult mice. Fourth, the inclusion of new mice in our experiments, which had differences in birth time and mother, litter, and experimental condition, add potential bias and confounding to our outcomes. Finally, mice in both baseline and anesthesia groups received 60% oxygen, leading to potential confounding influences caused by effects of released oxygen free radical. However, previous studies using the same anesthesia (3% sevoflurane plus 60% oxygen) also illustrated age-dependent anesthesia neurotoxicity,[14] so we used this established system to demonstrate age-dependent Tau metabolism and associated changes. In conclusion, our results indicate that higher brain Tau concentrations and lower activity of the mitochondria–ATP–Nuak1–Tau phosphorylation cascade in neonatal compared with adult mice may serve as an underlying mechanism of age-dependent Tau phosphorylation and cognitive impairment after administration of sevoflurane anesthesia in mice.

Research Support

Supported by National Institutes of Health grant No. HD086977 (Bethesda, Maryland; to Dr. Xie).

Competing Interests

Dr. Zhongcong Xie provides consulting service to Shanghai Jiaotong University (Shanghai, China), Tongji University (Shanghai, China), Baxter (Deerfield, Illinois; as an invited speaker) and Novartis (Basel, Switzerland). The other authors declare no competing interests.

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