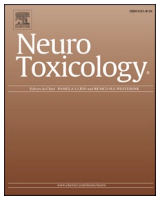
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Prolonged sevoflurane exposure induces exaggerated and persistent cognitive decline in old rats with pre-existing impairment by increasing hippocampal mitochondrial dysfunction and apoptosis

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ABSTRACT

Background: Postoperative neurocognitive disorders are common in older adults; whether prolonged volatile anesthesia causes persistent decline or amplifies preexisting vulnerability remains uncertain. We assessed whether sevoflurane exposure induces persistent cognitive impairment and hippocampal injury in young and aged rats, stratified by baseline cognitive status.

Methods: Female Wistar Hannover rats comprised young adults (10–12 weeks; n = 40) or aged (>24 months; n = 82). Aged rats were classified as cognitively intact (n = 47) or impaired (n = 35) after Morris Water Maze (MWM) pre-screening. Animals received 2% sevoflurane (SEVO+) or oxygen/air (SEVO−) for 3-h. Spatial memory was assessed by MWM probe trials (days 7, 30, and 90), with retraining. Subsets were sacrificed 24-h after exposure (n = 6/group) for histopathology and immunohistochemistry; remaining rats were evaluated after final behavioral analysis.

Results: SEVO caused transient memory impairment in young rats on day 7, resolving by day 30. In aged rats, effects were greater and more persistent, especially in cognitively impaired animals, with reduced target-

Abbreviations: **Bax**, Bcl-2–associated X protein; **Bcl-2**, B-cell lymphoma 2; **CA1,**, Cornu Ammonis 1; **Cl-Cas-3**, Cleaved caspase-3; **COX-IV,**, Cytochrome-c oxidase subunit 4; **Drp1,**, Dynamin-related protein 1; **GPx1,**, Glutathione Peroxidase 1; **HE,**, Hematoxylin Eosin; **Hsp,**, Heat-shock protein; **IHC**, Immunohistochemistry; **MWM,**, Morris water maze; **POCD,**, Postoperative cognitive dysfunction; **ROS**, Reactive Oxygen Species; **SEVO,**, Sevoflurane.

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quadrant preference, fewer crossings on days 7 and 30, and poor long-term retention. Acutely, SEVO increased hippocampal CA1 injury and elevated pro-apoptotic (Bax/Bcl-2, Cl-Cas-3) and mitochondrial/oxidative stress markers (Drp1, COX-IV, GPX1, Hsp60). Long-term, persistent CA1 pathology and elevated amyloid-β were most prominent in cognitively impaired aged rats.
Conclusions: Prolonged SEVO exposure was associated with brief cognitive disruption in young female adults but led to more persistent cognitive decline and hippocampal injury in older rats with pre-existing impairment, underscoring baseline cognitive vulnerability as a modifier of long-term anesthesia-associated neurocognitive outcomes and an important consideration in surgical/anesthetic risk management.

1. Introduction

Postoperative cognitive dysfunction (POCD) is a prevalent and serious complication among older adults, linked to diminished quality of life, prolonged hospitalizations, and higher rates of morbidity and mortality (Evered and Silbert, 2018). POCD is a neurological complication seen mostly in elderly patients after major surgery, marked by deficits in attention, learning, memory, and executive function. These clinical manifestations can lead to prolonged hospitalization, functional decline, and an elevated risk of long-term adverse outcomes. As the availability of surgical interventions for older adults broadens, the clinical significance of POCD has become increasingly prominent (Sun et al., 2024; Tawfik et al., 2025).

Surgery and anesthesia may lead to POCD, particularly deficits in memory, learning, attention, and executive function, often linked to elevated Alzheimer's disease-related proteins amyloid beta and phosphorylated tau (Jiang and Jiang, 2015; Chen et al., 2023). Moreover, anesthesia-induced cognitive impairment is associated with processes including neuroinflammation, oxidative stress, and cell death (Smith et al., 2024; Zhang et al., 2024). Research in rodents, including our previous studies (Demirgan et al., 2019, 2021, 2026; Kargı-Gemici et al., 2024) and other investigations (Xiao et al., 2016; Maloney et al., 2019), has shown that volatile anesthetics may cause lasting learning and memory deficits associated with brain tissue damage, particularly in the hippocampus.

Sevoflurane (SEVO) is among the most commonly used inhalational anesthetics in clinical practice because of its rapid onset, low blood-gas solubility, and favorable cardiovascular profile. Nonetheless, emerging experimental evidence suggests that it may exert neurotoxic effects, particularly in aged animal models (Peng et al., 2012; Wang et al., 2020; Chen et al., 2023). Specifically, SEVO exposure in older animals has been associated with impaired hippocampal function, linked to tau phosphorylation and mitochondrial dysfunction (Cascella and Bimonte, 2017; Zhang et al., 2024). It is also important to note that aging is associated with a natural decline across several cognitive domains (Zhang et al., 2025; Strzelczyk et al., 2026). Therefore, a major question is whether the cognitive decline observed in older patients after anesthesia is caused by the anesthetic drugs themselves or by age-related neurodegenerative changes. Animal studies allow researchers to explore this question under controlled conditions. Cognitive decline in humans typically begins after age 60, whereas rats are considered aged at 24 months or older and naturally exhibit reduced learning and memory abilities (Burke and Barnes, 2006; Gilbert et al., 2009). Studies have shown that aged rats take longer to locate the platform and spend less time in the target quadrant than younger counterparts during learning and memory tests (Cascella and Bimonte, 2017; Jia et al., 2023; Li et al., 2025). However, the long-term effects of prolonged anesthesia on brain tissue damage and cognition in older adults, particularly those with cognitive impairment, and whether neurotoxicity depends on age or dementia status, are not fully understood. Clinical concerns support the need for preoperative cognitive assessment and risk stratification among older patients (Brodier and Cibelli, 2021). Given clinical evidence that preoperative mild cognitive impairment (MCI) or dementia increases the risk of postoperative delirium and that postoperative delirium is associated with later diagnosis of MCI or dementia in elderly surgical patients (Sprung et al., 2017), we hypothesized that prolonged sevoflurane exposure would cause transient deficits in young adult rats but persistent hippocampal injury and spatial memory impairment in aged rats, particularly in those with pre-existing cognitive impairment.

In this study, aged female rats underwent pre-screening with the Morris Water Maze (MWM) and were classified into cognitively impaired and unimpaired subgroups, which were assessed for the effects of SEVO anesthesia on cognitive function in both early and late phases. Young, intact rats served as a young adult control group, and the effects of anesthesia were also examined in these animals. MWM testing was conducted over a three-month post-anesthesia period to monitor cognitive deficits. Additionally, brain tissues were examined using histopathological hematoxylin and eosin (HE) staining to assess hippocampal injury, and immunohistochemical analysis (Bax, Bcl−2, caspase−3, Drp1, COX-IV, GPx1, Hsp60, and amyloid-β) was used to evaluate neurodegeneration. Our findings show that young rats experienced only transient cognitive impairment after anesthesia, with recovery. In contrast, aged rats, particularly those with preexisting cognitive deficits, exhibited prolonged hippocampal vulnerability, persistent biomarker changes, and enduring cognitive dysfunction over the three months after SEVO exposure. Because male animals were not evaluated, the extent to which these observations generalize across sexes remains unknown and warrants future investigation. These results indicate that neurodegenerative effects induced by anesthesia are reversible in younger female subjects but may persist longer in aged, cognitively compromised individuals.

2. Material and methods

2.1. Ethics statement, animals, and experimental design

This study included 122 female Wistar Hannover rats; 82 were aged (over 24 months, weighing 400–450 g), and 40 were young adults (10–12 weeks, weighing 300–350 g). All rats were bred and aged in-house at BADABEM (Experimental Research and Skill Development Training Center in Istanbul), and all studies were conducted there. Rats were randomly assigned to groups and housed in pairs (two per cage) in polycarbonate Type 3 H cages (425 ×265 ×180 mm; PLEXX, The Netherlands) under controlled conditions (21 ± 1°C, 12:12-h light/dark cycle). Standard chow and water were provided ad libitum, with food pellets and 750 mL drinker cups placed on a stainless-steel wire grid. All procedures were approved by the Animal Ethics Committee of the University of Health Sciences, Bağcılar Training and Research Hospital, Istanbul, Türkiye (protocol number: 2018–07, January 29, 2018), and complied with the ARRIVE guidelines and the National Research Council's standards for animal care, thereby reducing the number of animals used and minimizing their suffering.

Only female rats were used to maintain sex consistency across groups and reduce sex-related biological variability, as the primary aim was to examine the effects of age, baseline cognitive status, and SEVO exposure. In this context, to assess cognitive competence prior to anesthesia, both young and aged rats were tested in the Morris Water Maze (MWM) in two phases: training (learning test, 4 consecutive days) and a probe trial (memory test, 24-h after last training). Cognitive classification was adapted from previous MWM-based studies in which aged rodents were

stratified according to baseline spatial learning or memory performance relative to young reference animals (Rowe et al., 1998; Gerstein et al., 2013; Yang et al., 2015). In the present study, classification was based on the percentage of time spent in the target quadrant during the baseline probe trial; the 30% cutoff was selected because it exceeded chance performance (25%) and fell within the lower range of young adult performance in our dataset (*detailed in the results section*). Preliminary cognitive evaluation showed no impairment in young rats; therefore, cognitive stratification was applied only to aged rats, who were classified as cognitively intact (n = 47) or cognitively impaired (n = 35). Afterward, rats were randomly divided into six groups: ***(1) Young Intact SEVO***− (cognitively intact, no anesthesia, n = 20); ***(2) Young Intact SEVO***+ (cognitively intact, received SEVO, n = 20); ***(3) Old Intact SEVO***− (cognitively intact, no anesthesia, n = 24); ***(4) Old Intact SEVO***+ (cognitively intact, received SEVO, n = 23); ***(5) Old Impair SEVO***− (cognitively impaired, no anesthesia, n = 16); ***(6) Old Impair SEVO***+ (cognitively impaired, received SEVO, n = 19).

One day after anesthesia, 6 rats from each group were randomly selected for histopathological and immunohistochemical analyses. The remaining rats underwent cognitive testing using the MWM protocol. Memory probe trials occurred on days 7 and 30 post-anesthesia. Short- and long-term cognitive effects of SEVO were assessed with training on days 31–32 and probes on days 33 and 90, and with training on days 91–92 and a probe test on day 93. The experimental design is schematically shown in Fig. 1.

2.2. Sevoflurane exposure

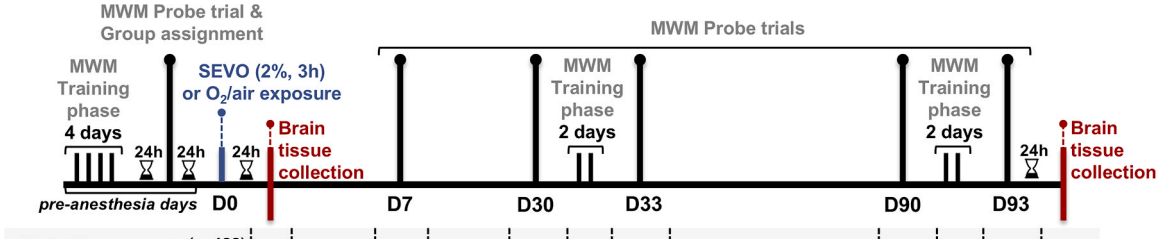
Sevoflurane (SEVO, 2%) anesthesia in a 50/50% oxygen/air mixture was administered for 3-h in a temperature-controlled Plexiglas chamber (13 ×13 ×20 cm). Each rat was placed individually in the chambers during SEVO exposure to avoid inter-animal effects. The chamber temperature was maintained at 35–37°C throughout the exposure. The 3-h exposure duration was chosen to model a prolonged single anesthetic exposure and aligns with preclinical studies that used 3-h sevoflurane exposure to evaluate anesthesia-associated cognitive impairment in aged rodents (Ma and Chen, 2021; Xie et al., 2021). Rats in unanesthetized groups were exposed to a 50% oxygen/air mixture for 3-h under the same chamber conditions, without SEVO administration. Gas concentrations (SEVO, O_2, CO_2) were continuously monitored with an infrared analyzer (Datex-Ohmeda S/5 Avance, GE Healthcare). Gas flow was maintained at 5–7 L/min to keep end-tidal CO_2 between 1 and 2 mmHg. Respiratory rate, skin color, and depth of anesthesia were monitored throughout the exposure period, and respiratory rate was measured manually by an observer as breaths per minute.

2.3. Morris water maze experiments

As previously described by Demirgan et al. (2021), with minor modifications, animals were tested by an experimenter blinded to the experimental groups using the MWM to assess spatial learning and memory. Tests were conducted in a circular black tank (150 cm in diameter, 60 cm deep) filled with opaque water and divided into four quadrants. A transparent platform (10 ×10 cm) was submerged 1 cm below the water surface and placed 30 cm from the wall. The platform location for each rat was randomly determined, and its position was kept constant in all trials. For example, if the platform location is southeast (SE), trial experiments were conducted by starting the rat from positions except for S, E, and SE. Indirect lighting (50 ± 10 lx), fixed visual cues, and a surrounding curtain minimized distractions during behavioral monitoring. MWM experiment details are available in Supplementary Fig.S1 *and Table.S1*. All MWM tests were video recorded (The related MWM videos are included in the Supplementary Materials).

The behavioral protocol began with a four-day acquisition phase conducted six days before anesthesia exposure, during which rats completed four daily trials. Each trial began by releasing rats from semi-random points into a pool to find a hidden platform within 60-sec. If the animal found the platform, it stayed for 20-sec; if not, it was guided to the platform and rested for 30-sec. Escape latency was measured each time. A probe trial took place 24-h after the last acquisition trial to assign groups; in this trial, the platform was removed, and rats swam freely for 60-sec while their time in each quadrant, crossings over the former platform spot, and swim paths were recorded. One day after the group assignment, the anesthesia exposure protocol was implemented, and the experimental start time was designated Day 0 (D0). Subjects were tested repeatedly in the MWM at D7, 30, 31–33, and 90–93. Memory probe trials occurred on days 7 and 30 post-anesthesia. Additionally, short- and long-term cognitive effects of SEVO were assessed with training on days 31–32 and probes on days 33 and 90, and with training on days 91–92 and a probe test on day 93.

All trials were video-recorded and subsequently analyzed using ImageJ (version 1.52 u, NIH, USA) and the AnimalTracker plugin (Gulyás et al., 2016). Videos were converted using Weeny Free Video Converter version 2.0. The swimming routes of the rats were recorded in



Wistar Hannover rats (n=122)	n	n	n	n	n	n	n
Young Intact SEVO− (n=20)	6	14	14	14	14	14	14
Young Intact SEVO+ (n=20)	6	12	12	12	12	12	12
Old Intact SEVO− (n=24)	6	18	17	17	17	14	14
Old Intact SEVO+ (n=23)	6	16	16	16	16	14	14
Old Impair SEVO− (n=16)	6	10	10	10	10	10	10
Old Impair SEVO+ (n=19)	6	13	13	13	12	12	12

Fig. 1. Schematic of the experimental design and timeline. Wistar Hannover rats (n = 122) were grouped by age (young/old) and cognitive status (intact/impaired), then assigned to sevoflurane (SEVO, 2%, 3 h) or O_2/air exposure. All rats completed 4 days of Morris Water Maze (MWM) training prior to anesthesia. Twenty-four hours after probe trials, they received SEVO or O_2/air; some were sacrificed 24-h later for brain tissue collection. The first probe trial was performed on D7. Long-term cognition was assessed with repeated MWM probes, including retraining on D30–D33 and D90–D93, followed by memory tests. Final brain samples were collected 24-h after the probe on D93. The number of animals analyzed at each time point is indicated in the timeline below.

both the learning phase and the probe trial. Quantitative behavioral parameters (escape latency, time spent in the target quadrant, swimming speed, and number of crossings of the former platform location) were extracted and statistically analyzed.

2.4. Tissue preparation

For histopathological and immunohistochemical analyses, some animals were sacrificed 24-h after the anesthesia protocol (n = 6/group), and the remaining animals were sacrificed on day 94 at the end of the experimental protocol (shown in Fig. 1). On Day 1, these animals were used to assess early molecular effects of SEVO or O_2 exposure. In addition, the remaining animals assigned to behavioral testing were sacrificed after the final cognitive assessment sessions. Following sacrifice, all animals were subjected to transcardial perfusion with ice-cold phosphate-buffered saline (PBS; 0.01 M, pH7.4), and the brain was removed from the cranial cavity. The brain was fixed in 10% neutral-buffered formalin for 24-h. Coronal brain sections ~2–4 mm posterior to the optic chiasma, corresponding to the anterior hippocampus, were obtained. Paraffin embedding was performed, and four coronal sections per brain were cut at a thickness of 6μm using a rotary microtome (Leica RM2255). The samples were then prepared for histopathological evaluation using hematoxylin and eosin (HE) staining and for immunohistochemical analyses targeting specific molecular markers.

2.5. Histopathological analysis

Brain tissue sections embedded in paraffin were stained with hematoxylin and eosin (HE) for morphological analysis. The 6μm sections, prepared by rotary microtome, were deparaffinized, rehydrated, and stained. The hippocampal cornu ammonis (CA) 1 region was examined for neuronal damage, indicated by pyknosis, cytoplasmic shrinkage, or nuclear condensation. All histological assessments were performed at 400 × magnification by a single blinded pathologist using light microscopy (Nikon Eclipse Ni-u; Tokyo-Japan). Staining intensity was measured using Fiji software (ImageJ v2), and the results are presented as mean optical density (MOD), calculated as log(max intensity/mean intensity).

2.6. Immunohistochemical analysis

Paraffin-embedded brain sections were deparaffinized in xylene, rehydrated through a graded ethanol series, and subjected to antigen retrieval. To inhibit endogenous peroxidase activity, sections were treated with 10% hydrogen peroxide in methanol for 10 min at 37°C. Nonspecific binding was minimized with a blocking solution. Sections were incubated with primary antibodies (for Bax, Bcl−2, caspase−3, amyloid-β, COX-IV, Drp1, GPx1, and Hsp60 levels) and then with appropriate peroxidase-labeled secondary antibodies. Dilutions and code/brand information for antibodies and antigen retrieval methods are provided in Table 1. A polymer-based detection method was used for signal amplification (Ultravision LP Detection System, TL−125-HD, Thermo-Scientific, Fremont, California). Antigen-antibody reactions were visualized with the 3,3′-diaminobenzidine (DAB) chromogen, and the sections were counterstained with Mayer hematoxylin. Finally, the sections were dehydrated through graded ethanol solutions (80–100%) and coverslipped. The hippocampal region was assessed, using light microscopy (Nikon Eclipse Ni-u; Tokyo-Japan) at 400 × magnification, by two blinded pathologists. Both cytoplasmic and nuclear staining were considered positive. Staining intensity was measured using Fiji software (ImageJ v2), and the results are presented as mean optical density (MOD), calculated as log(max intensity/mean intensity). To quantify data, three non-overlapping images of the hippocampal CA1 region were acquired from each animal for each staining analysis. The MOD values from these three images were averaged to produce a single value per animal.

2.7. Statistical analysis

Statistical analyses were conducted using GraphPad Prism software (version 10.2.2; SanDiego-USA). All results were presented as mean ± standard error of the mean (SEM). Data normality was assessed using the Shapiro–Wilk test. MWM learning trial results (over 2–4 days), were evaluated using a repeated-measures two-way ANOVA. Post-exposure comparisons were performed using two-way ANOVA with SEVO exposure (SEVO−/SEVO+) and cognitive status (Young Intact, Old Intact, Old Impair) as between-subjects factors. Significant effects or interactions were followed by Tukey's multiple-comparisons post hoc test. ANOVA outcomes are reported with F values, degrees of freedom, *P* values, and partial eta squared (ηp^2). Statistical significance was set at $P < 0.05$. A *P* value of < 0.05 was considered statistically significant.

3. Results

During the experimental period, a total of 10 rats died across the study groups. On Day 0 (during anesthesia), mortality was recorded in the Young Intact SEVO+ group (n = 2) and the Old Intact SEVO+ group (n = 1). During follow-up, one animal in the Old Intact SEVO− group died on Day 30 (n = 1). An additional death occurred in the Old Impair SEVO+ group between Days 33 and 90 (n = 1). Further mortality was observed between Days 90 and 93 in the Old Intact SEVO− group (n = 3) and the Old Intact SEVO+ group (n = 2).

3.1. Baseline MWM screening grouped aged rats as cognitively intact or impaired

As shown in Fig. 2A, the behavioral protocol began with a four-day acquisition phase conducted six days before anesthesia exposure, followed by a probe trial 24-h after the final acquisition session to assign the groups. Both young and aged rats reduced escape latency time during the acquisition phase, but aged rats learned the submerged platform location more slowly ($P = 0.0189$, Fig. 2B-i). In the probe trial, aged animals showed greater variability in target-quadrant time ($P < 0.0001$). In the baseline probe trial, the lower range of young adult target-quadrant performance was close to 30%: three young rats showed the lowest value of 28.33%. This value was reported only to describe the young reference range; cognitive stratification was applied only to aged rats. Aged rats were stratified into *Old Intact* and *Old Impair* subgroups

Table 1
Antibodies used for immunohistochemistry (IHC) analysis.

Antibody	Host	Dilution	Incubation	Code	Company	Antigen retrieval buffer
Anti-Bax	Rabbit	1:250	Overnight / 4°C	A00183	Boster Bio.	Citrate (pH 6.0)
Anti-Bcl−2	Rabbit	1:1000	1.5-h / 37°C	A00040–2	Boster Bio.	Citrate (pH 6.0)
Anti-Caspase−3	Rabbit	1:100	1.5-h / 37 °C	PB9188	Boster Bio.	Citrate (pH 6.0)
Anti-Amyloid-β	Rabbit	1:500	Overnight / 4°C	PB9091	Boster Bio.	Citrate (pH 6.0)
Anti-COX-IV	Rabbit	1:100	Overnight / 4°C	A05442–1	Boster Bio.	Citrate (pH 6.0)
Anti Drp1	Rabbit	1:150	Overnight / 4°C	A00556–2	Boster Bio.	Tris-EDTA (pH 8.0)
Anti-GPx1	Rabbit	1:250	Overnight / 4°C	PB9203	Boster Bio.	Citrate (pH 6.0)
Anti-Hsp60	Mouse	1:250	Overnight / 4°C	M01280	Boster Bio.	Tris-EDTA (pH 8.0)

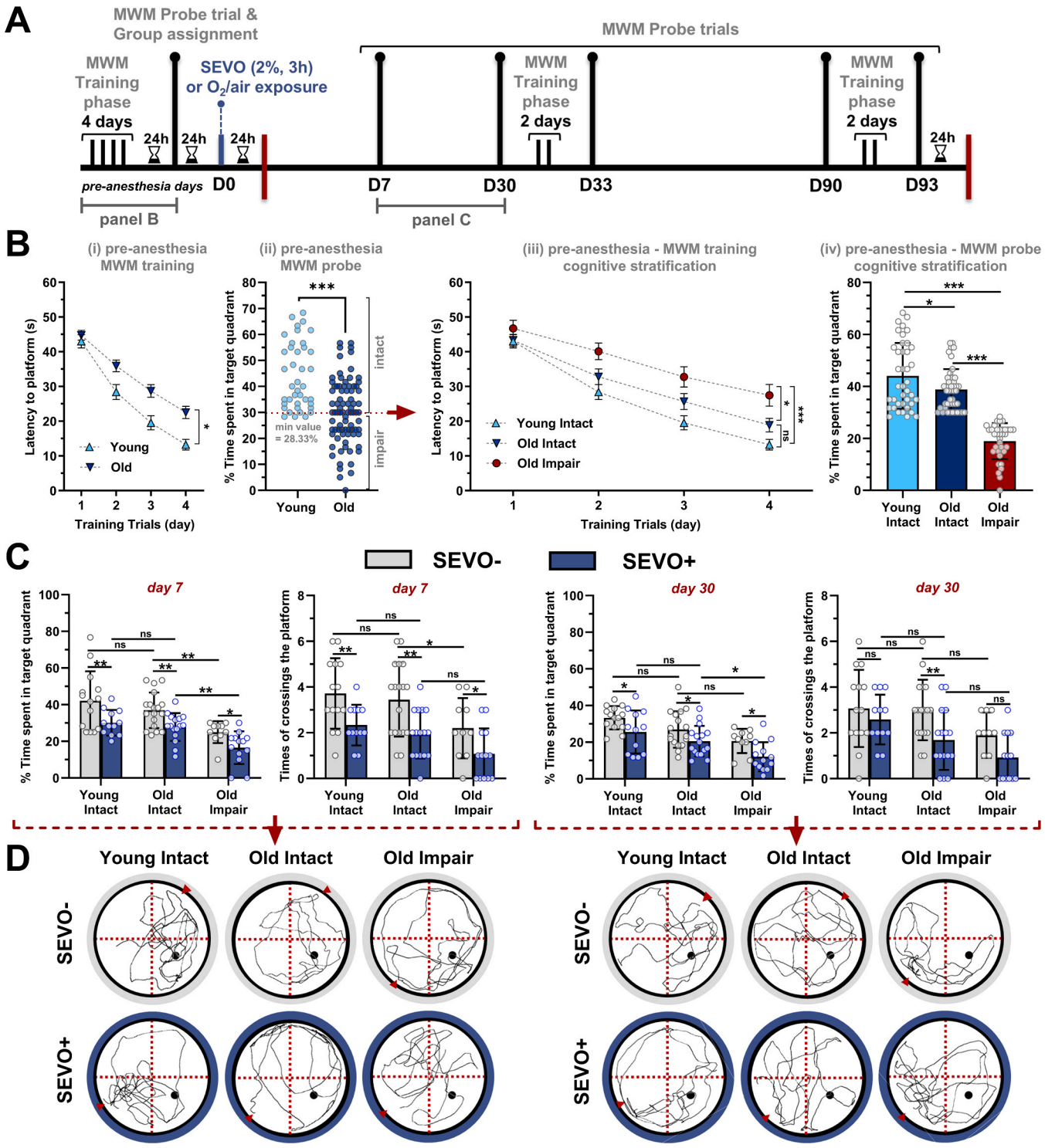


Fig. 2. Effects of sevoflurane exposure on spatial learning and memory in young and old rats, assessed with MWM tests. (A) Timeline and design. (B) Pre-SEVO group assessment: aged rats were categorized as "*Old Intact*" or "*Old Impair*" based on time spent in the target quadrant. The left panels show learning curves (i) and time distributions in the target quadrant (ii), including the threshold for distinguishing intact from impaired aged rats. The right panels compare escape latency (iii) and probe (iv) results for *Young Intact*, *Old Intact*, and *Old Impair* groups. (C) Probe trial performance at D7 and D30 under SEVO− (50/50% O_2/air for 3-h) and SEVO+ (2% sevoflurane, 3-h) conditions: bar graphs show time in the target quadrant and platform crossings. (D) Representative swim paths during probe trials at D7 and D30. Data are presented as mean±SEM; individual data points represent single animals. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; *ns*, not significant.

based on a predefined cutoff value for target-quadrant time (30%, Fig. 2B-ii). The basis for the 30% cutoff is as follows: Gerstein et al. (2013) used target-quadrant preference, whereas Rowe et al. (1998) and Yang et al. (2015) classified aged rats using cohort-specific criteria relative to young controls rather than a universal cutoff. In our cohort, young rats had a mean target-quadrant time of approximately 44%; only three young rats were slightly below 30% (28.33%), while all others were ≥ 30%. Thus, 30% was selected because it was above the chance level (25% chance of being found in each quadrant) and captured the lower end of young performance.

When MWM data were re-evaluated across the three groups by cognitive classification, the differences among the groups were determined as follows: *Impair* rats learned the platform location ($P < 0.0001$ and $P = 0.0193$, respectively, Fig. 2B-iii) and spent less time in the target-quadrant than both *Young* and *Old Intact* rats ($P < 0.0001$ for both, Fig. 2B-iv), confirming successful group assignment prior to SEVO exposure (group effects for latency graph: F_{2476}=21.57, $P < 0.0001$, ηp^2=0.08; group effects for spent time in target-quadrant: F_{2476}=71.68, $P < 0.0001$, ηp^2=0.55). Additionally, young and old intact rats exhibited similar performance in MWM learning trials ($P > 0.05$), but in the probe test, the young rats spent more time in the target quadrant than the old rats ($P < 0.0001$) (Fig. 2B).

3.2. Sevoflurane caused transient memory impairment in young rats but persistent deficits in aged rats

After allocation to SEVO+ or SEVO− conditions, spatial memory was assessed with probe trials (Fig. 2A). At D7 (Fig. 2C), target-quadrant time and platform-site crossings were significantly affected by both SEVO exposure ($F_{1,77}$=19.64, $P < 0.0001$, ηp^2=0.20; $F_{1,77}$=20.96, $P < 0.0001$, ηp^2=0.21, respectively) and cognitive status ($F_{2,77}$=15.50, $P < 0.0001$, ηp^2=0.29; $F_{2,77}$=7.35, $P = 0.0012$, ηp^2=0.16, respectively). Compared with the *Young Intact SEVO−* group, the *Young Intact SEVO+* group exhibited reduced target-quadrant time (30.13%±2.02, $P = 0.0030$) and fewer former platform-site crossings (2.33 ± 0.26, $P = 0.0088$). Similarly, SEVO exposure impaired probe-trial performance in *Old Intact* rats, resulting in lower target-quadrant occupancy (27.79%±1.89, $P = 0.0092$) and fewer platform-site crossings (1.94 ± 0.27, $P = 0.0012$). The most pronounced effects of SEVO were observed in *Old Impair* rats, which showed reduced target-quadrant preference (16.54%±2.49, $P = 0.0471$) and fewer platform-site crossings (0.92 ± 0.29, $P = 0.0444$) compared with their non-anesthetized counterparts.

At D30, significant effects of SEVO exposure and cognitive status remained for target-quadrant time ($F_{1,76}$=14.90, $P = 0.0002$, ηp^2=0.16; $F_{2,76}$=13.49, $P < 0.0001$, ηp^2=0.26, respectively) and platform-site crossings ($F_{1,76}$=10.35, $P = 0.0019$, ηp^2=0.12; $F_{2,76}$=7.53, $P = 0.0010$, ηp^2=0.17, respectively). In *Young Intact* rats, most anesthesia-related deficits had resolved by D30, with only target-quadrant time remaining reduced in the SEVO+ group (25.56%±3.38, P = 0.0277). In contrast, *Old Intact* rats continued to show impaired probe performance despite partial recovery, as reflected by reduced target-quadrant time (19.25%±2.08, P = 0.0415) and platform-site crossings (1.69 ± 0.33, P = 0.0044). Likewise, *Old Impair* rats displayed persistent cognitive impairment, with a sustained reduction in target-quadrant time in the SEVO+ group (13.20%±2.28, $P = 0.0306$), although the difference in platform-site crossings was no longer significant.

Between-group comparisons further indicated that probe-trial performance did not differ between the Young Intact and Old Intact groups at D7 under SEVO exposure (target-quadrant: $P = 0.8117$; crossings: $P = 0.7082$). Likewise, at D30, target-quadrant time was comparable between these groups under both SEVO− ($P = 0.1123$) and SEVO+ ($P = 0.2999$) conditions. In contrast, *Old Intact* rats consistently outperformed *Old Impair* rats. At D7, significant differences were observed for both probe-trial measures (SEVO+ groups, target-quadrant: P = 0.0090; SEVO− groups, target-quadrant: P = 0.0094; crossings: P = 0.0471). This effect persisted at D30, with the *Old Intact SEVO+* group maintaining superior target-quadrant performance relative to the *Old Impair SEVO+* group (P = 0.0295).

Representative swim-path traces (Fig. 2D) corroborated the quantitative data, showing reduced search accuracy in the *Old Impair SEVO+* group at both the D7 and D30 assessments. *Young Intact* animals typically exhibited focused searching near the former platform site, whereas *Old Impair* animals, especially following SEVO exposure, displayed broader, non-targeted search patterns during both early and late probe trials, indicating diminished spatial memory precision.

3.3. Sevoflurane impaired long-term retention in cognitively impaired aged rats

To assess whether prolonged SEVO exposure causes long-term spatial learning and memory deficits, rats underwent repeated retraining and probe trials over three months (Fig. 3A). Retraining occurred on days 31–32, with a probe trial on day 33. Long-term retention was tested with a probe trial on day 90. Rats then received a second retraining on days 91 and 92, followed by a probe on day 93.

As shown in Fig. 3B, during the initial retraining phase (days 31 and 32), both *Young* and *Old Intact* rats showed a decrease in latency. In contrast, cognitively impaired rats exhibited relearning deficits, as indicated by higher escape latencies. Specifically, the *Old Impair SEVO−* group recorded a latency of 32.98 ± 4.35-sec compared to 14.96 ± 2.35-sec in the *Old Intact SEVO−* group ($P = 0.0018$). Similarly, the *Old Impair SEVO+* group showed a latency of 37.33 ± 3.67-sec versus 16.88 ± 2.48-sec in the *Old Intact SEVO+* group ($P < 0.0001$) on day 32. At D33, two-way ANOVA showed that cognitive-status significantly affected rat performance on target-quadrant time ($F_{2,76}$=14.46, $P < 0.0001$, ηp^2=0.28) and platform-site crossings ($F_{2,76}$=9.54, $P = 0.0002$, ηp^2=0.20). No significant within-group differences were observed following SEVO exposure. However, under the *SEVO+* condition, a significant difference was detected between the *Old Intact* and *Old Impair* groups at D33 (target-quadrant: $P = 0.0215$; crossings: $P = 0.0064$).

At D90, two-way ANOVA showed that target-quadrant time was significantly affected by both SEVO exposure ($F_{1,70}$=5.14, $P = 0.0265$, ηp^2=0.07) and cognitive-status ($F_{2,70}$=12.50, $P < 0.0001$, ηp^2=0.26), whereas platform-site crossings were significantly affected only by cognitive-status ($F_{2,70}$=13.05, $P < 0.0001$, ηp^2=0.27). Long-term retention (Fig. 3C) remained low in old, cognitively impaired rats, and SEVO exposure further aggravated this deficit: the *Old Impair SEVO+* group showed decreased target-quadrant time (15.70%±1.98, $P = 0.0367$) than the *Old Impair SEVO−* group. Representative swim traces (Fig. 3D) supported these quantitative results. While *Young* and *Old Intact* animals showed more focused searching around the former platform region, old, impaired rats, particularly after SEVO exposure, exhibited more dispersed, non-target exploration patterns, consistent with impaired spatial memory precision.

As shown in Fig. 3E, during the second retraining phase (D91 and D92), the *Old Impair SEVO+* group (32.35 ± 3.78-sec, $P = 0.0027$) and the *Old Impair SEVO−* group (28.55 ± 3.85-sec, $P = 0.0014$) continued to show higher latencies than the *Old Intact* groups. At D93, two-way ANOVA revealed a significant effect of cognitive status on both target-quadrant time ($F_{2,70}$=24.10, $P < 0.0001$, ηp^2=0.41) and platform-site crossings ($F_{2,70}$=13.65, $P < 0.0001$, ηp^2=0.28). The probe trial results showed that memory retrieval remained compromised in the *Old Impair SEVO+* group; these rats exhibited lower target-quadrant time (19.17% ±1.60, $P = 0.0003$) and fewer platform crossings (0.83 ± 0.27, $P = 0.0126$) than the *Old Intact SEVO+* groups. In contrast, differences between SEVO− and SEVO+ conditions were minimal or nonsignificant in *Young Intact* and *Old Intact* animals at this late postoperative stage.

To rule out locomotor impairment as the primary driver of differences in MWM performance, swim speed was analyzed as an additional locomotor parameter independent of cognitive performance (Supplementary Fig. S2). SEVO exposure did not significantly affect swim speed in the *Young Intact*, *Old Intact*, or *Old Impair* groups at days 7, 30, or 90. However, aged rats, particularly the *Old Impair* group, swam more slowly than *Young Intact* rats, indicating an age- and baseline cognitive status-related reduction in general locomotor performance. These additional data support the interpretation that the long-term behavioral changes observed after SEVO exposure mainly reflect cognitive impairment rather than a general locomotor deficit.

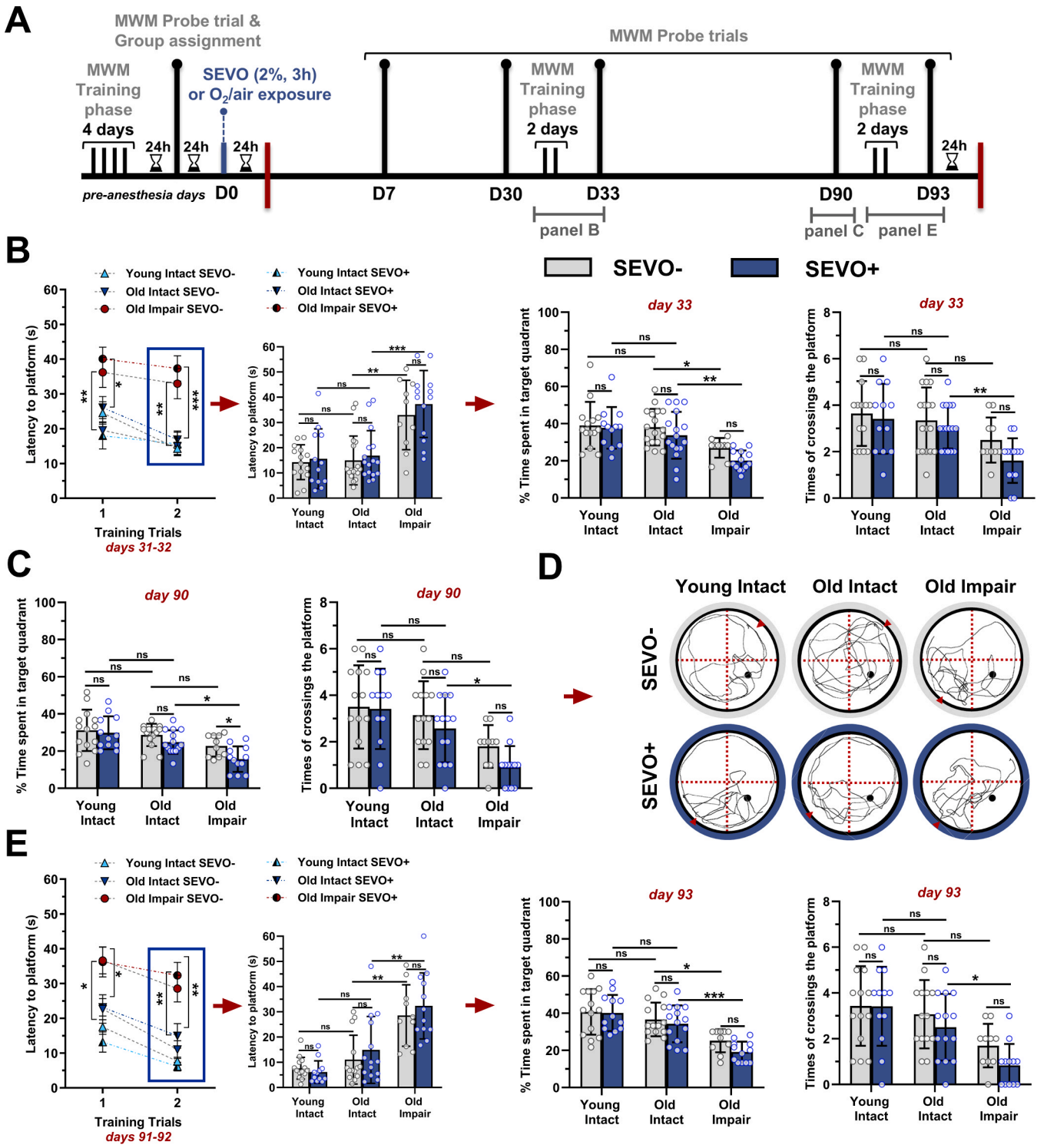


Fig. 3. Effects of sevoflurane exposure on long-term retention in young and old rats, assessed with MWM tests. (A) Timeline and design. Spatial learning and memory were assessed over three months in *Young Intact*, *Old Intact*, and *Old Impair* rats using retraining and probe tests under SEVO− (50/50% O_2/air for 3-h) and SEVO+ (2% sevoflurane, 3-h) conditions. (B) First relearning performance, including escape latency (days 31–32) and probe trial (day 33). (C) Long-term retention at day 90, shown as % time in the target quadrant and number of platform-site crossings, and (D) representative swim paths from the probe test. (E) Second relearning performance, including escape latency (days 91–92) and probe trial (day 93). Data are presented as mean ± SEM with individual data points representing single animals. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; *ns*, not significant.

3.4. Early apoptotic/neurodegenerative signaling after sevoflurane exposure is followed by persistent hippocampal apoptotic damage and enhanced amyloid-β, predominantly in aged, cognitively impaired rats

To determine whether prolonged SEVO exposure produces acute damage in the hippocampus, depending on age and pre-existing cognitive status, the hippocampal CA1 region was analyzed on post-anesthesia day 1 (Fig. 4). Two-way ANOVA showed that HE-detected CA1 damage was significantly affected by both SEVO exposure ($F_{1,30}$=51.14, $P < 0.0001$, ηp^2=0.63) and cognitive status ($F_{2,30}$=10.33,

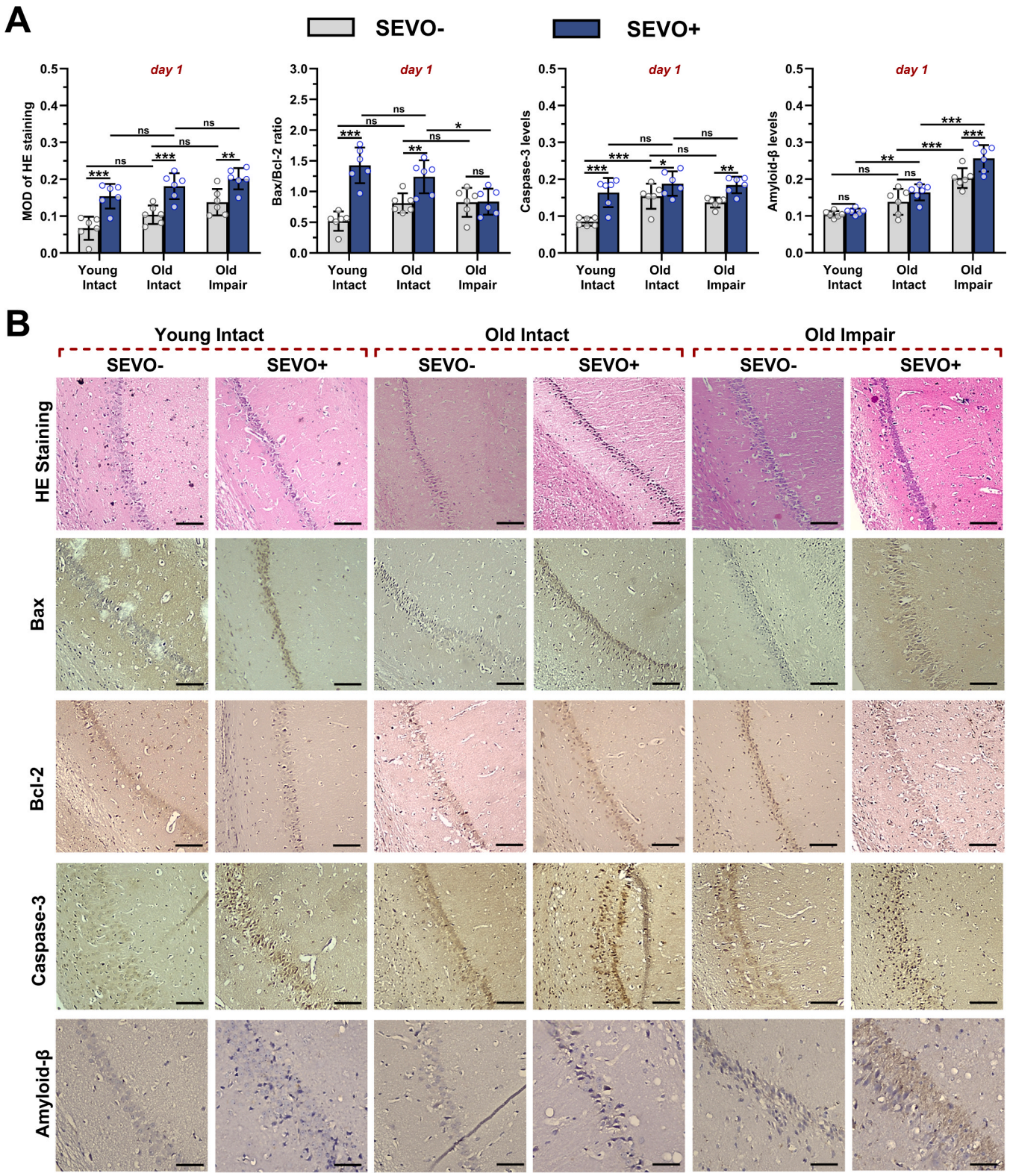


Fig. 4. Early apoptotic and neurodegenerative alterations in the hippocampal CA1 region one day after sevoflurane exposure. *Young Intact*, *Old Intact*, and *Old Impair* rats were exposed to 50/50% O_2/air (3-h, SEVO−) or 2% sevoflurane (3-h, SEVO+), and hippocampal tissue was collected on post-anesthesia day 1. (A) Damage in the CA1 region was quantified using hematoxylin–eosin (HE) staining and by measuring the Bax/Bcl−2 ratio, active caspase−3, and amyloid-β levels. (B) Representative images are shown for HE and immunohistochemical staining (400 × magnification, scale-bar = 50 μm). Data are presented as mean±SEM, with individual data points representing single animals. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; *ns*, not significant.

$P = 0.0004$, $\eta p^2 = 0.41$). However, no significant total-group interaction was observed ($F_{2,30} = 0.41$, $P = 0.6689$, $\eta p^2 = 0.03$). SEVO increased the number of damaged cells in the CA1 region, particularly in the *Young Intact* group (2.29-fold, $P < 0.0001$), followed by the *Old Intact* (1.74-fold, $P = 0.0002$) and *Old Impair* (1.46-fold, $P = 0.0017$) groups. The Bax/Bcl−2 ratio was significantly affected by SEVO exposure ($F_{1,30} = 35.43$, $P < 0.0001$, $\eta p^2 = 0.54$), with a significant total-group interaction ($F_{2,30} = 11.84$, $P = 0.0002$, $\eta p^2 = 0.44$). This SEVO-related pro-apoptotic shift was primarily driven by increased Bax/Bcl−2 ratios in the *Young Intact* (2.75-fold, $P < 0.0001$) and *Old Intact* (1.53-fold, $P = 0.0026$) groups. Active caspase−3 levels were also markedly affected by SEVO exposure ($F_{1,30} = 33.39$, $P < 0.0001$, $\eta p^2 = 0.53$) and cognitive-status ($F_{2,30} = 9.33$, $P = 0.0007$, $\eta p^2 = 0.38$), with no significant interaction between these factors ($F_{2,30} = 2.05$, $P = 0.1466$, $\eta p^2 = 0.12$). Within-group comparisons showed that SEVO exposure increased active caspase−3 levels in the *Young Intact* (1.93-fold, $P < 0.0001$), *Old Intact* (1.22-fold, $P = 0.0407$), and *Old Impair* (1.34-fold, $P = 0.0061$) groups. Besides, amyloid-β levels were significantly influenced by SEVO exposure ($F_{1,30} = 11.64$, $P = 0.0019$, $\eta p^2 = 0.28$) and cognitive status ($F_{2,30} = 70.59$, $P < 0.0001$, $\eta p^2 = 0.82$), whereas their interaction was not significant ($F_{2,30} = 2.50$, $P = 0.0995$, $\eta p^2 = 0.14$). SEVO exposure increased amyloid-β levels only in the *Old Impair* group (1.26-fold, $P = 0.0010$), and under SEVO+ conditions, significant differences were observed between the *Young Intact* and *Old Intact* groups ($P = 0.0036$) and between the *Old Intact* and *Old Impair* groups ($P < 0.0001$).

To assess whether early SEVO-associated alterations persist, hippocampal CA1 tissue was examined 3 months after anesthesia (Fig. 5). Two-way ANOVA revealed significant effects of SEVO exposure ($F_{1,70} = 105.70$, $P < 0.0001$, $\eta p^2 = 0.60$), cognitive status ($F_{2,70} = 128.10$, $P < 0.0001$, $\eta p^2 = 0.79$), and their interaction ($F_{2,70} = 17.46$, $P < 0.0001$, $\eta p^2 = 0.33$) on HE-detected CA1 damage at day 94. SEVO exposure did not alter CA1 damage in *Young Intact* animals, whereas it produced significant increases in the *Old Intact* (1.65-fold, $P < 0.0001$) and *Old Impair* (1.58-fold, $P < 0.0001$) groups. Bax/Bcl−2 ratio and active caspase−3 levels were significantly influenced by both SEVO exposure ($F_{1,70} = 4.32$, $P = 0.0413$, $\eta p^2 = 0.06$; $F_{1,70} = 4.77$, $P = 0.0323$, $\eta p^2 = 0.06$, respectively) and cognitive status ($F_{2,70} = 12.57$, $P < 0.0001$, $\eta p^2 = 0.26$; $F_{2,70} = 21.02$, $P < 0.0001$, $\eta p^2 = 0.38$, respectively). Under SEVO+ conditions, both markers differed between the *Young Intact* and *Old Intact* groups ($P = 0.0058$ and $P = 0.0025$, respectively). Amyloid-β levels were also significantly affected by SEVO exposure ($F_{1,70} = 9.39$, $P = 0.0031$, $\eta p^2 = 0.12$) and cognitive status ($F_{2,70} = 33.74$, $P < 0.0001$, $\eta p^2 = 0.49$). Notably, amyloid-β levels also remained higher on day 94, with the high elevation observed in the *Old Impair SEVO+* group (1.27-fold, $P = 0.0088$) compared with the *Old Impair SEVO−* group, consistent with long-lasting neurodegenerative-like signaling.

Overall, prolonged SEVO exposure induced acute hippocampal CA1 injury accompanied by apoptotic and neurodegenerative-like signaling, with significant effects of SEVO exposure and cognitive status on several markers. Although early apoptotic markers increased across groups, amyloid-β elevation was most pronounced in the *Old Impair* group. On day 94, persistent CA1 damage and sustained amyloid-β elevation were predominantly observed in aged animals, particularly in the Old Impair SEVO+ group, suggesting that pre-existing cognitive vulnerability contributes to long-term hippocampal susceptibility after SEVO exposure.

3.5. *Sevoflurane induces early mitochondrial and oxidative-stress responses in the hippocampus and long-term dysregulation, predominantly in aged, cognitively impaired rats*

To further characterize early molecular responses associated with prolonged SEVO exposure, age, and pre-existing cognitive status, we examined hippocampal CA1 immunoreactivity of markers related to mitochondrial function and stress responses (COX-IV, Drp1, GPx1, Hsp60) on post-anesthesia day 1 (Fig. 6). Two-way ANOVA indicated that COX-IV levels were significantly affected only by SEVO exposure ($F_{1,30} = 17.02$, $P = 0.0003$, $\eta p^2 = 0.36$), with a selective increase in the *Young Intact SEVO+* group compared with *Young Intact SEVO−* controls (1.66-fold, $P = 0.0002$). Drp1 and Hsp60 levels were significantly influenced by both SEVO exposure ($F_{1,30} = 20.21$, $P < 0.0001$, $\eta p^2 = 0.40$; $F_{1,30} = 7.66$, $P = 0.0096$, $\eta p^2 = 0.20$, respectively) and cognitive status ($F_{2,30} = 38.20$, $P < 0.0001$, $\eta p^2 = 0.72$; $F_{2,30} = 14.66$, $P < 0.0001$, $\eta p^2 = 0.49$, respectively). Drp1 increased in the *Young Intact SEVO+* (1.40-fold, $P = 0.0309$) and *Old Intact SEVO+* (1.67-fold, $P < 0.0001$) groups, whereas Hsp60 increased only in the *Old Intact SEVO+* group compared to *Old Intact SEVO−* (1.33-fold, $P = 0.0094$). Within SEVO+ animals, the acute effect resulted in the *Old Intact* rats having higher Drp1 levels ($P = 0.0070$) than the *Young Intact*, and the *Old Impair* animals having lower Hsp60 levels than the *Old Intact* ($P = 0.0007$). GPx1 levels were affected by SEVO exposure ($F_{1,30} = 37.71$, $P < 0.0001$, $\eta p^2 = 0.56$), cognitive status ($F_{2,30} = 23.45$, $P < 0.0001$, $\eta p^2 = 0.61$), and their interaction ($F_{2,30} = 17.03$, $P < 0.0001$, $\eta p^2 = 0.53$). GPx1 increased in the *Young Intact SEVO+* (1.78-fold, $P < 0.0001$) and *Old Intact SEVO+* (1.37-fold, $P < 0.0001$) groups compared with their respective *SEVO−* controls. Among SEVO− animals, GPx1 levels were higher in *Old Intact* than *Young Intact* rats ($P = 0.0009$) and further elevated in *Old Impair* compared with *Old Intact* rats ($P = 0.0002$).

To determine whether early SEVO-induced changes persist, hippocampal CA1 tissue was examined 3 months after anesthesia (Fig. 7). Two-way ANOVA showed that COX-IV ($F_{2,70} = 14.15$, $P < 0.0001$, $\eta p^2 = 0.29$), Drp1 ($F_{2,70} = 51.74$, $P < 0.0001$, $\eta p^2 = 0.60$), and GPx1 ($F_{2,70} = 4.04$, $P = 0.0220$, $\eta p^2 = 0.10$) levels were significantly affected only by cognitive-status. COX-IV levels were higher in *Old Intact* than *Young Intact* rats under both SEVO− ($P = 0.0200$) and SEVO+ ($P = 0.0028$) conditions, whereas Drp1 remained elevated in *Old Impair* compared with *Old Intact* rats in both conditions ($P < 0.0001$). GPx1 levels did not significantly differ across groups. In contrast, Hsp60 levels were affected by both SEVO exposure ($F_{1,70} = 4.06$, $P = 0.0476$, $\eta p^2 = 0.05$) and cognitive status ($F_{2,70} = 6.08$, $P = 0.0037$, $\eta p^2 = 0.15$). Following SEVO exposure, Hsp60 increased significantly in the *Old Impair* group ($P = 0.0352$), and among SEVO− animals, Hsp60 differed between the *Old Intact* and *Old Impair* groups ($P = 0.0162$).

Prolonged SEVO exposure triggered early mitochondrial and oxidative-stress responses in the hippocampal CA1 region, as reflected by changes in COX-IV, Drp1, GPx1, and Hsp60 levels. These acute responses were most pronounced in Young Intact and Old Intact animals, whereas long-term alterations appeared to be driven mainly by cognitive status rather than persistent SEVO effects. At the late time point, sustained Drp1 elevation in Old Impair animals, together with SEVO-induced Hsp60 upregulation in this group, indicates persistent mitochondrial-stress vulnerability in cognitively impaired aged rats.

4. Discussion

Postoperative cognitive dysfunction (POCD) remains a major clinical concern in aging populations, particularly as surgical interventions become increasingly common among elderly individuals (Arefayne et al., 2023). Although sevoflurane (SEVO, a volatile anesthetic) is considered hemodynamically stable and clinically advantageous, accumulating experimental evidence indicates that its neurobiological impact varies by age and cognitive context (Bittner et al., 2011; Neag et al., 2020). The present study shows that a single, prolonged exposure to SEVO (2% for 3-h) produces age- and vulnerability-dependent neurocognitive consequences. Using pre-screening with the Morris Water Maze (MWM), aged rats were stratified into cognitively intact and cognitively impaired subgroups, enabling the observation that baseline cognitive function significantly influences post-anesthesia outcome trajectories. Young adult rats exhibited transient memory impairment at postoperative day 7, with substantial recovery by day 30, whereas aged rats, particularly those with pre-existing impairment, showed deficits that persisted up to 90 days after exposure. This behavioral pattern was accompanied by hippocampal CA1 pathology and a biomarker profile

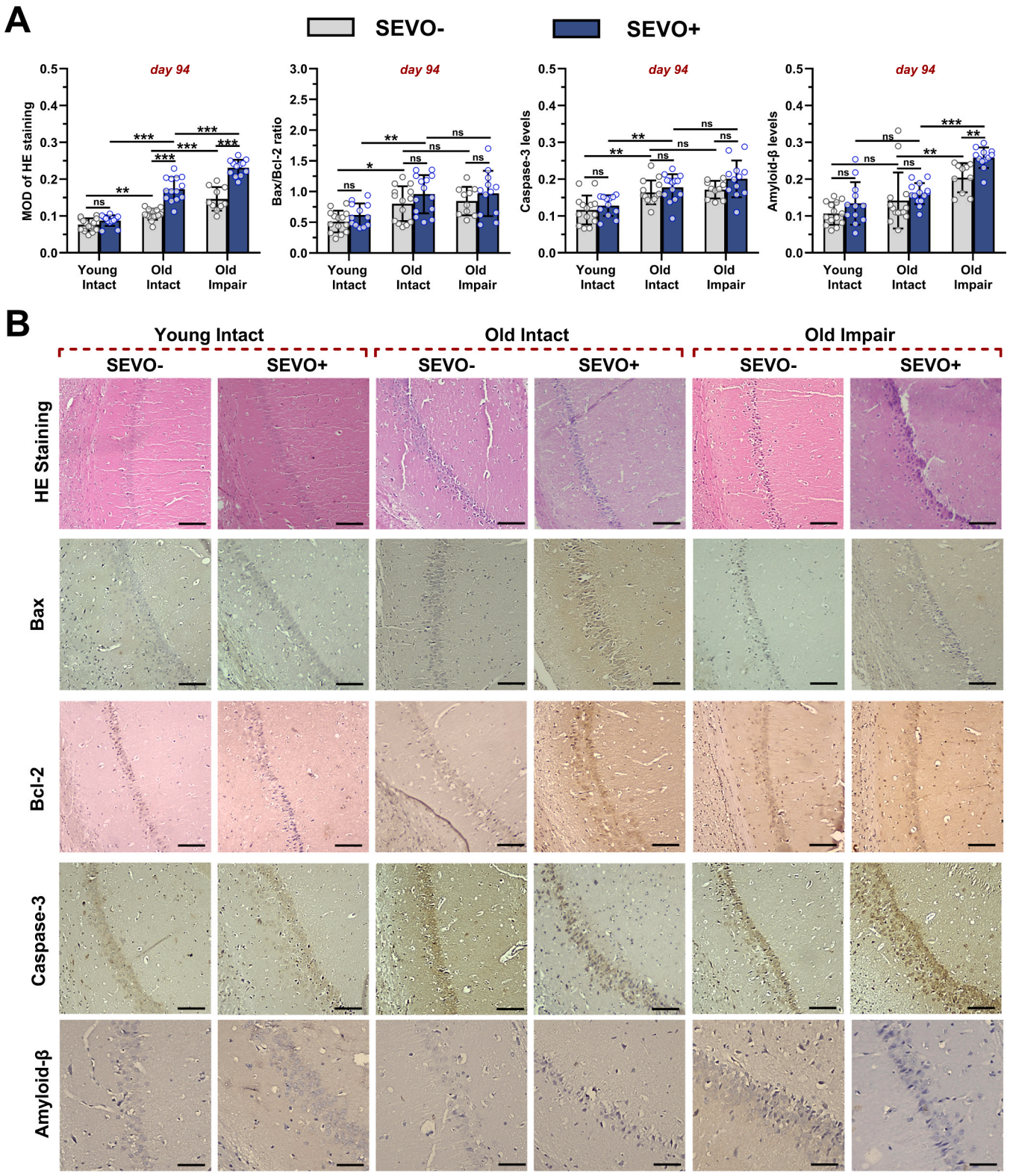


Fig. 5. Persistent apoptotic damage and amyloid-β accumulation in the hippocampal CA1 region three months after sevoflurane exposure. *Young Intact*, *Old Intact*, and *Old Impair* rats were exposed to 50/50% O_2/air (3-h, SEVO−) or 2% sevoflurane (3-h, SEVO+), and hippocampal tissue was collected on post-anesthesia day 94. (A) Damage in the CA1 region was quantified using hematoxylin–eosin (HE) staining and by measuring the Bax/Bcl−2 ratio, active caspase−3, and amyloid-β levels. (B) Representative images are shown for HE and immunohistochemical staining (400 × magnification, scale-bar = 50 μm). Data are presented as mean±SEM, with individual data points representing single animals. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; *ns*, not significant.

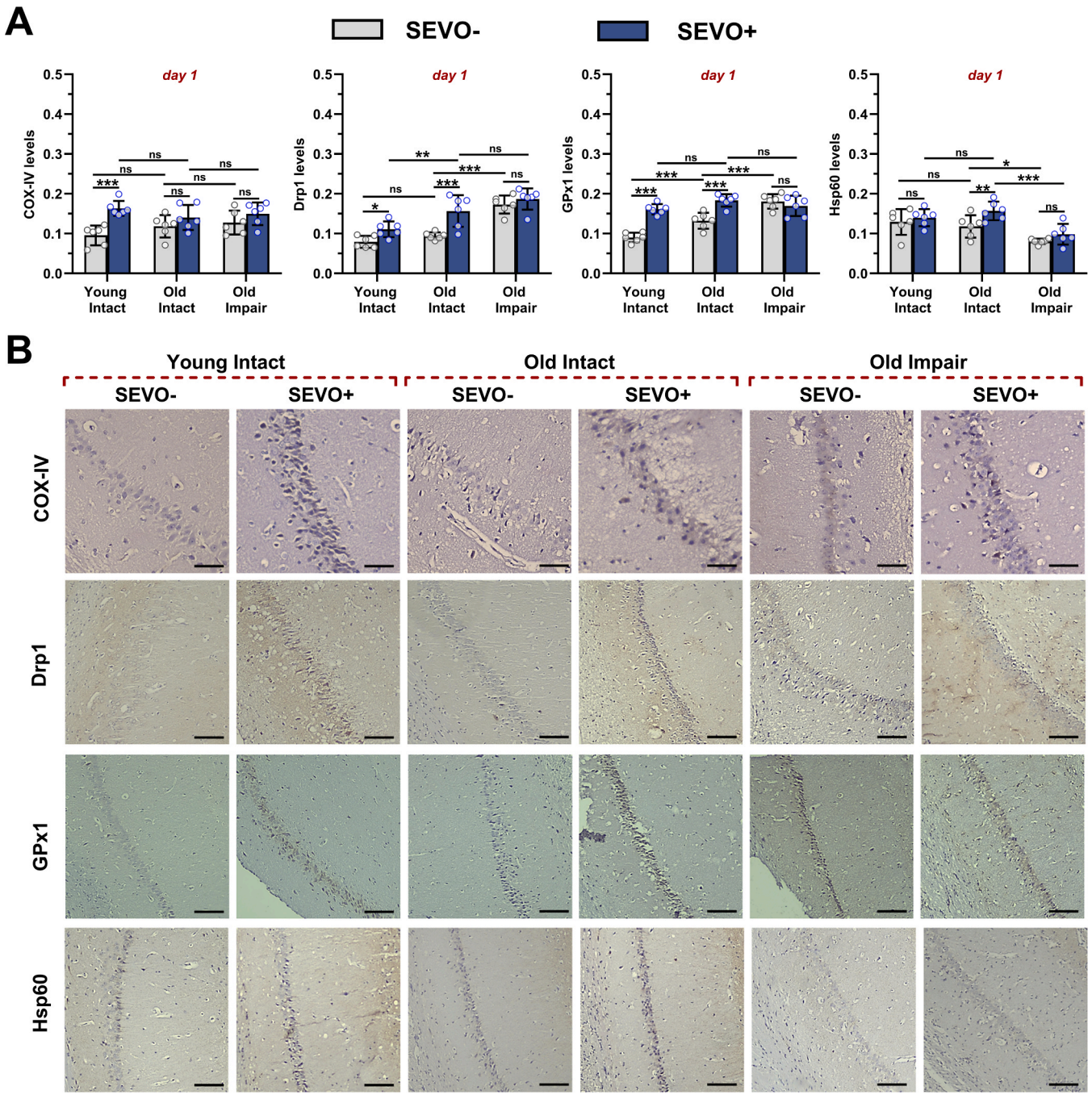


Fig. 6. Early alterations in mitochondrial dynamics, oxidative stress, and heat shock response markers in the hippocampal CA1 region one day after sevoflurane exposure. *Young Intact*, *Old Intact*, and *Old Impair* rats were exposed to 50/50% O_2/air (3-h, SEVO−) or 2% sevoflurane (3-h, SEVO+), and hippocampal tissue was collected on post-anesthesia day 1. (A) Quantitative analysis of cytochrome c oxidase subunit IV (COX-IV), dynamin-related protein 1 (Drp1), glutathione peroxidase 1 (GPX1), and heat shock protein 60 (Hsp60) levels by immunohistochemical staining. (B) Representative immunohistochemical staining images of COX-IV, Drp1, GPX1, and Hsp60 (400 × magnification, scale-bar = 50 μm). Data are presented as mean±SEM, with individual data points representing single animals. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; *ns*, not significant.

consistent with early apoptosis and sustained mitochondrial/oxidative stress dysregulation, with the most durable amyloid-β signal emerging in cognitively impaired aged animals.

A well-documented feature of neurocognitive aging is heightened variability among individuals; some aged animals perform as well as younger peers, whereas others show pronounced impairments. These differences are thought to reflect variations in cognitive reserve and the integrity of hippocampal networks (Gallagher et al., 2006; Bizon et al., 2009). Our pre-screening approach operationalized this concept by stratifying aged rats into cognitively intact and impaired subgroups before exposure. After SEVO, the old, impaired subgroup showed consistent worsening across probe trials (D7, D30) and the longest retention deficit (D90). This finding aligns with clinical observations that pre-existing cognitive vulnerability and frailty increase perioperative neurocognitive disorder risk (Berger et al., 2018; Evered and Silbert, 2018) and supports the idea that anesthesia may act as a second hit in susceptible brains rather than a sole cause of decline (Eckenhoff et al., 2020).

Young rats exposed to SEVO showed a quantifiable short-term reduction in target-quadrant preference and platform-site crossings on

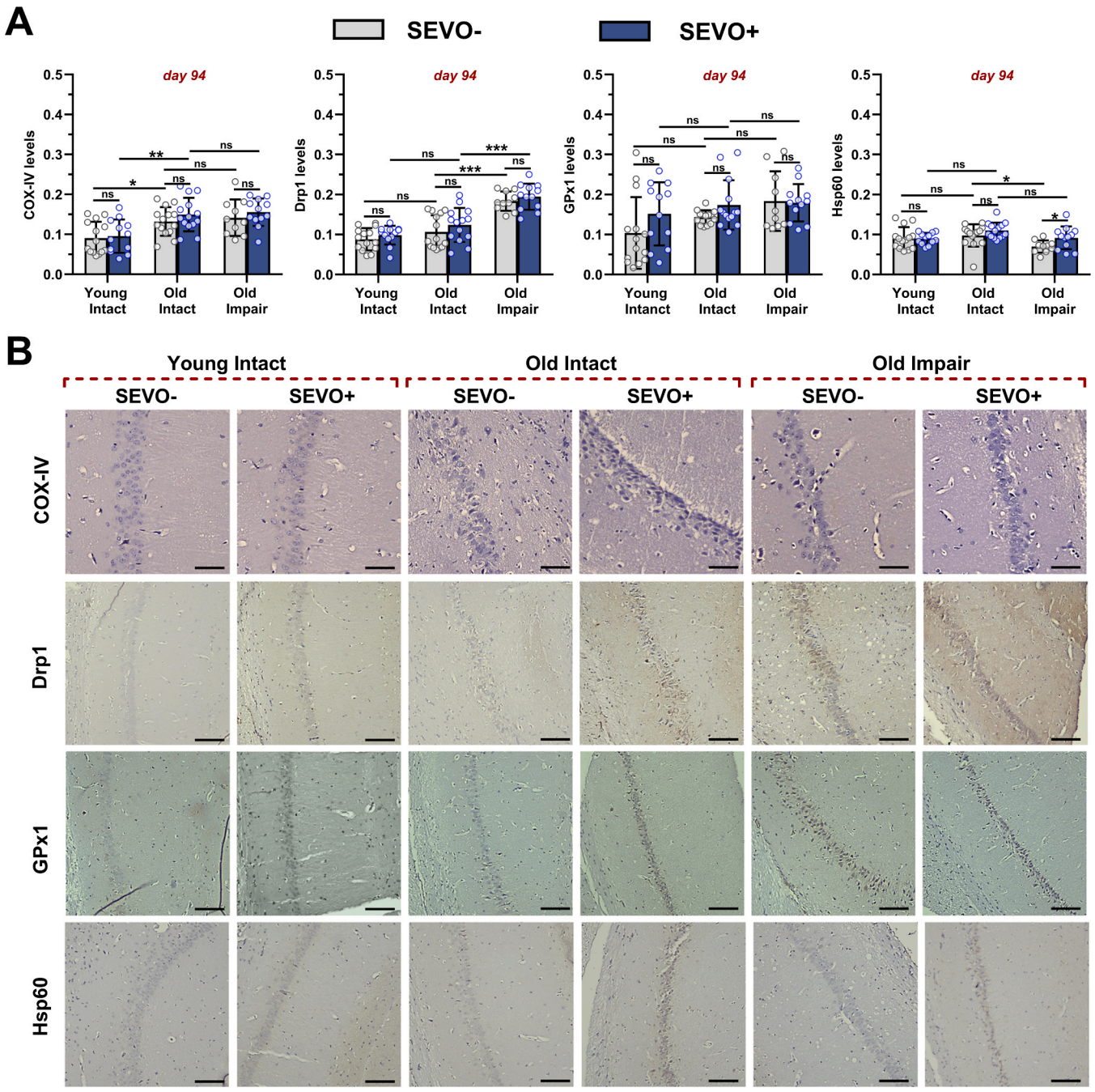


Fig. 7. Long-term alterations in mitochondrial dynamics, oxidative stress, and heat shock response markers in the hippocampal CA1 region three months after sevoflurane exposure. *Young Intact*, *Old Intact*, and *Old Impair* rats were exposed to 50/50% O_2/air (3-h, SEVO−) or 2% sevoflurane (3-h, SEVO+), and hippocampal tissues were collected on day 94 after anesthesia. (A) Quantitative analysis of cytochrome c oxidase subunit IV (COX-IV), dynamin-related protein 1 (Drp1), glutathione peroxidase 1 (GPX1), and heat shock protein 60 (Hsp60) levels in the CA1 region by IHC. (B) Representative immunohistochemical staining images of COX-IV, Drp1, GPX1, and Hsp60 (at 400 × magnification, scale-bar = 50 μm). Data are presented as mean±SEM, with individual data points representing single animals. $^{*}P < 0.05$, $^{***}P < 0.001$; *ns*, not significant.

day 7; however, these effects were largely resolved by day 30. This pattern of recovery aligns with studies indicating that non-aged brains can compensate for anesthetic stress through intact synaptic plasticity, antioxidant mechanisms, and mitochondrial quality control (Wu et al., 2019; Eckenhoff et al., 2020; Zhang et al., 2024). Although marked early CA1 histopathology and pro-apoptotic signaling were evident in young subjects at day 1, these molecular alterations did not result in persistent cognitive deficits (Wu et al., 2019; Wang et al., 2021). One possible explanation is that the young hippocampus mounts a more effective adaptive response to anesthetic stress, potentially mediated by antioxidant and mitochondrial stress-response mechanisms (Massaad and Klann, 2011; Zhang et al., 2024).

Behavioral outcomes varied across subgroups and were consistent with the threshold and cognitive-reserve models, suggesting that lower baseline cognitive performance may be associated with greater and more prolonged effects after exposure to a stressor (Stern, 2002). Consistent with this view, early probe deficits observed after SEVO in young animals were largely resolved by about day 30, whereas the aged, cognitively impaired subgroup showed deficits that persisted through day 90. The results indicate that younger brains may recover more

readily from anesthesia, whereas older brains have greater difficulty in restoring hippocampal function. This supports the clinical view that cognitive changes after surgery depend on multiple factors, with existing cognitive impairment being a key predictor of poor outcomes (Rundshagen, 2014; Evered et al., 2018).

Early post-SEVO-exposure changes in the hippocampal CA1 region reflect an acute stress response that triggers apoptosis, including increased Bax/Bcl−2 ratios and caspase activation, consistent with prior findings that volatile anesthetics can activate cell-death pathways (Pape et al., 2006; Zhou et al., 2016). Additionally, inhalational anesthetics have been found to alter amyloid-β production and aggregation in cellular and animal studies (Xie and Xu, 2013; Jiang and Jiang, 2015). The pronounced increase in amyloid-β immunoreactivity in aged, cognitively impaired individuals suggests that SEVO may worsen hippocampal degeneration when resilience is low. Elevated amyloid-β could be a cause, effect, or indicator of shared stress pathways (Xie and Xu, 2013; Sun, 2020). The presence of early apoptotic markers alongside amyloid-β changes is consistent with a two-stage model in which an early injury response may contribute to longer-term network dysfunction and cognitive impairment (Wu et al., 2019; Wang et al., 2021).

Mitochondria are essential for neuronal survival and hippocampal plasticity, so even mild disruptions in mitochondrial function can impair learning and memory (Reddy, 2008; Swerdlow, 2018). In our study, SEVO exposure produced an early rise in COX-IV, Drp1, and GPx1 levels, most clearly in young and old intact rats, consistent with an acute metabolic and oxidative-stress response. Because COX-IV (cytochrome c oxidase or complex IV) is commonly used as an index of inner-mitochondrial-membrane respiratory capacity and mitochondrial content, its selective elevation in young intact SEVO+ animals may reflect an early compensatory "bioenergetic upshift" to meet increased ATP demand in CA1 after prolonged anesthesia (Hawong et al., 2015). Drp1 (dynamin-related protein 1) is a core driver of mitochondrial fission, and excessive fission is linked to mitochondrial fragmentation and synaptic vulnerability in neurodegenerative conditions (Wang et al., 2009). Notably, Drp1 remained elevated in old, impaired animals on day 94, even after apoptotic markers had normalized, suggesting that persistent alterations in mitochondrial dynamics may contribute to sustained cognitive deficits, even when acute apoptotic signaling is less pronounced. This finding aligns with reports that SEVO disrupts mitochondrial quality control and mitophagy in aged rats (Chen et al., 2020) and with broader reviews linking SEVO-related perioperative neurocognitive disorders to mitochondrial and oxidative stress pathways (Wang et al., 2021). Oxidative-stress markers support this interpretation. SEVO can disturb redox balance and increase ROS, and the early GPx1 increase likely reflects a compensatory antioxidant response (Chen et al., 2013). GPx1 (glutathione peroxidase 1) is a selenium-dependent enzyme found in both the cytosol and mitochondria that detoxifies hydrogen peroxide and lipid hydroperoxides using reduced glutathione (GSH), helping limit ROS-driven mitochondrial and synaptic vulnerability (Ursini et al., 2016; Brigelius-Flohé and Flohé 2020). Accordingly, acute GPx1 upregulation in intact rats after SEVO likely represents an adaptive attempt to buffer transient peroxide overload (Cobley et al., 2018). Although GPx1 differences were not prominent at the late time point, elevated Hsp60 in old, impaired animals suggests ongoing mitochondrial stress. Mechanistically, Hsp60 (heat-shock protein 60) is a mitochondrial matrix chaperonin that supports folding and handling of imported-proteins and is often considered as a node of mitochondrial proteostasis that rises with mitochondrial stress and oxidative injury; thus, sustained Hsp60 alterations would be consistent with a prolonged mitochondrial unfolded-protein/proteostasis burden rather than a short-lived redox perturbation (Haynes and Ron, 2010; Quirós et al., 2015). Taken together, the early COX-IV/GPx1 increases in intact animals may represent an adaptive surge capacity, whereas the persistence of elevated Drp1 (and accompanying proteostasis stress signatures) in old, impaired animals suggests a maladaptive remodeling of mitochondrial dynamics that can plausibly undermine synaptic maintenance and memory circuits long after the anesthetic exposure.

These findings have clinical translational relevance. First, they reinforce the view that older adults with pre-existing cognitive impairment may be especially vulnerable to prolonged anesthetic exposure (Rundshagen, 2014; Wu et al., 2019). Second, they indicate that anesthesia-induced neurotoxicity is not invariably permanent but rather influenced by baseline brain health and cognitive reserve (Feinkohl et al., 2017). Clinically, these insights underscore the importance of conducting preoperative cognitive assessments and risk stratification in elderly patients (Brodier and Cibelli, 2021). Identifying those with diminished cognitive reserve may inform decisions about anesthetic selection, exposure duration, and postoperative monitoring protocols (Feinkohl et al., 2017). Although SEVO remains widely used because of its favorable pharmacokinetic profile, limiting prolonged exposure in populations at elevated risk may be advisable (Wu et al., 2019). Nevertheless, caution should be exercised when applying findings from rodent models directly to humans. Clinical investigations have yielded inconsistent results on the long-term cognitive effects of anesthesia alone (Seitz et al., 2011), and it is important to consider that surgical inflammation, underlying systemic diseases, and comorbidities may interact with anesthetic effects in complex ways. More recent clinical studies also underscore the importance of postoperative cognitive monitoring in older adults. In a double-blind randomized trial of elderly cancer patients, Guo et al. (2020) reported postoperative cognitive dysfunction in both sevoflurane- and propofol-based anesthesia groups at 7 days and 3 months, although sevoflurane did not significantly increase POCD compared with propofol. Similarly, Li et al. (2021) found no clear difference between intravenous and volatile anesthesia in delayed neurocognitive recovery after laparoscopic abdominal surgery, while postoperative inflammatory activity was associated with cognitive outcome. A recent network meta-analysis of randomized trials in elderly noncardiac surgery patients further showed that the incidence of POCD varies across anesthetic drug regimens, supporting the concept that anesthetic exposure interacts with patient vulnerability and perioperative factors rather than acting as an isolated determinant Zeng et al. (2023).

Consequently, prolonged exposure to SEVO temporarily impairs cognition in young adults but causes lasting cognitive decline and hippocampal damage in older rats with preexisting impairments. However, the results are limited by the use of an anesthesia-only model, which excludes surgery-related factors such as trauma or inflammation, and by the inclusion of female Wistar rats only. This sex-specific study design restricts the generalizability of the findings to females and precludes conclusions about potential sex-dependent differences in cognitive and neuropathological responses to SEVO exposure. Therefore, all findings should be interpreted in the context of the female population only, and future studies incorporating age-matched male cohorts will be necessary to determine whether the observed effects are reproducible across sexes and more broadly applicable. Another limitation is that aged rats were classified using baseline MWM performance, and MWM was also used for post-SEVO assessment. Thus, differences between *Old Intact* and *Old Impair* groups may partly reflect baseline learning differences or repeated-testing effects, in addition to SEVO exposure. Future studies should use an independent test for cognitive pre-screening. Despite these constraints, the findings convey a coherent biological message. Prolonged SEVO exposure induced an early hippocampal disturbance, characterized by activation of stress-related and apoptotic pathways alongside a mitochondrial–redox imbalance. Importantly, however, the long-term neurocognitive outcome seems to be shaped most strongly by age and, crucially, by baseline vulnerability (cognitive reserve). Although this anesthesia-only model doesn't replicate the entire perioperative setting, it suggests that age and baseline cognitive vulnerability should be considered when evaluating potential neurocognitive risks associated with prolonged anesthetic exposure.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Compliance with Ethical Standards

All animal care and experiments were carried out in accordance with ARRIVE guidelines and the National Research Council's Guide for the Care and Use of Laboratory Animals. The investigation was approved by the Animal Ethics Committee of Health Sciences University, Bağcılar Training and Research Hospital, Istanbul/Türkiye (approval protocol number: 2018–07, January 29, 2018). This article does not contain any studies with human participants.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.neuro.2026.103538.

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