Olfactory bulbectomy leads to prolonged induction phase of sevoflurane anesthesia in rats

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Abstract

The effect of olfactory bulb lesions on the induction time of sevoflurane has never been studied. We aimed to investigate this issue. In this study, we found that the volume of olfactory bulbs and the pore of the fila olfactoria were significantly lower with the fibrosis of olfactory bulbs in animals subjected to olfactory bulbectomy. Volatile anesthetics induction times were measured in all groups. Prolonged induction was observed in olfactory bulbectomy group. It was concluded that increased induction times of sevoflurane may be due to the olfactory bulb lesion.

Key words: anesthesia; fila olfactoria; induction time; nose-to-brain delivery; olfactory bulb; olfactory bulbectomy; sevoflurane; transport pathway; volatile anesthetics

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Introduction

Volatile anesthetics have been widely used over decades in the clinical practice of anesthesiology.[1-3] They have been the mainstay of general anesthetics for millions of patients each year.[4] There have some benefits from volatile anesthesia over intravenous anesthesia, e.g. the ability to continuously monitor alveolar concentration, and potential organ protective properties. Volatiles are important agents in anesthesia practice.

Sevoflurane [2,2,2-trifluoro-1-(trifluoromethyl) ethyl fluoromethyl ether], as a volatile anesthetics, has been extensively employed for induction and maintenance of general anesthesia, because it has ideal pharmacokinetic properties and few adverse effects. It is an important volatile anesthetic with anti-inflammatory and neuroprotective effects.[5]

The main goal of anesthesiology is to achieve the best level of analgesia and rapid recovery of consciousness after anesthesia.[6] The preservation of spontaneous breathing during general anesthesia with anesthetic gases is practiced by many anesthesiologists.[6] The human brain has important anatomic structures.[7] One of these structures is the olfactory bulb (OB). The olfactory function requires intact olfactory and cortical structures, which may be destructed in some pathologic conditions and during cranial or transnasal surgical approaches to the anterior skull base.

Currently, coronavirus disease 2019 (COVID-19) is an important global problem.[8,9] Millions of people worldwide have been infected with the COVID-19 virus, and anosmia after COVID-19 infection is the leading cause of olfactory dysfunction in adults, accounting for up to 40% of all cases.[10] These patients with olfactory dysfunction after this infection have undergone surgical procedures under inhalational sevoflurane anesthesia. Interestingly, olfactory dysfunction or anosmia has been observed in many patients with COVID-19 infection. The clinical pharmacokinetic outcomes for inhaled anesthetic drugs in an anosmic patient after COVID-19 infection may affect systemic plasma concentrations of sevoflurane. The effect of sevoflurane may be changed by anosmia or olfactory dysfunction after COVID-19 infection, and this undesired pharmacokinetic profile of sevoflurane in anosmic patients after COVİD-19 infection will lead to diminished concentration in the target brain area. In this pandemic, elucidation of the mechanisms of altered induction time of sevoflurane seems to be imperative.

Olfactory bulb lesions (OBL) in experimental animal studies may also lead to olfactory dysfunction. Olfactory bulbectomy (OBX) may cause changes and dysfunction of olfactory cells.[11] The effect of OB injury on the induction time of sevoflurane has never been studied. The effect of sevoflurane was tested after OBL.

Methods

Animals

This study was reviewed by the Institutional Animal Care and Use Committee/Animal Experimentation Committee of Ataturk University (No. 2010/9, on October 22, 2010). All experiments were designed and reported according to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines.[12] A total of 29 female adult Wistar rats, 10–12 months old, weighing 260–310 g were used. The rats were obtained from Ataturk University. Animal models are

essential new pharmacotherapies.[13] Sex differences can be seen in animal studies. In this study, female rats were used because Ruda-Kucerova et al.[13] reported the advantage of female rats. The rats were obtained from Ataturk University and housed in single cages under standard conditions (23 ± 3°C).

Animals were randomly divided into three groups by blinded experimenters. 0.2 mL/kg of the anesthetic combination (ketamine HCl, 150 mg/1.5 mL; xylazine HCL, 30 mg/1.5 mL; and distilled water, 1 mL) was subcutaneously injected before surgery to reduce animals' pain as reported in the study by Kanat et al.[14] The animals were fixed in a stereotactic frame (Stoelting Co., Wood Dale, IL, USA). Volatile anesthetic sevoflurane was administered using a modified anesthesia unit. Sevoflurane (Sevorane®, Abbott, North Chicago, IL, USA) was given via a face mask. In clinical practice, estimation of the depth of anesthesia is often quantified as minimum alveolar concentration or measured variables such as bispectral index scores. In this study, sevoflurane was administered using a calibrated vaporizer at a concentration of 1 minimum alveolar concentration with an expiratory fraction of 2.5%.[15] The presence of the toe-pinch reflex was sought, but animals had to be able to tolerate ventilation without distress. The depth of anesthesia was tested every 30 minutes in case of respiratory distress as previously used in a study.[16] If the rat was in an abnormal posture (four limbs upward) and failed to actively return to a normal posture; this was used to define the induction time of anesthesia.[17] There were nine rats in the control group (n = 9). No intervention was performed in the control group. In other animals, the frontal portions of the skull were shaved, and a burr hole over the midline of the frontal bone above the OBs was removed.[18] and the OBs were ablated by aspiration. In this technique, only one burr-hole on the midline of the frontal bone, 7 mm anterior to the bregma, was made. The animals in the sham group (n = 8) only received frontal burr holes at the level of the OB, and OBL was not made by clamping the OB. Immediately after exposure to the OBs, the burr holes were plugged and the skin incision was sutured. However, in the third group (n = 12), mechanic compressions of OB were applied by using a micro clamp, and bilateral OBL was performed, and during this procedure, care was taken to avoid damage to the frontal cortex. After this procedure, the animals of the study group were expected to be anosmic or hyposmic. The animals were observed for 8 weeks and then decapitated. Following the burr hole by using an appropriately sized facemask, before decapitation, the induction measures of sevoflurane were remeasured. If the rat was in an abnormal posture (four limbs upward) and failed to actively return to a normal posture; this was used to define the anesthesia induction time.[16]

Evaluation of the induction phase of sevoflurane in the experimental groups

The sevoflurane (Sevorane®, Abbott) was applied by a face mask and its induction time of sevoflurane was obtained. Mean induction time was assessed based on the time interval between the placement of the face mask and the loss of eyelash reflex.[14] As the mean induction time was found to be 135 seconds, the higher value of 165 seconds was accepted as a late induction time. The instant when the first signs of inactivity appeared was recorded to estimate the depth of anesthesia. We measured the time until the first inactivity signs such as no awakening reaction to sevoflurane anesthesia (1 point); the disappearance of the movement of limbs (2 points), lips (3 points), and whiskers (4 points); closing of the palpebral fissures (5 points); and loss of light reflex (6 points) via pain stimulation, and these examination parameters constituted the unconsciousness index of sevoflurane anesthesia. The sum of the criteria values was 21 points. A value higher than 15 points was accepted as the beginning of anesthesia. Unconsciousness index values and induction times were compared. A pain stimulator applied pressure (kg/point) on the tails of the rats. 250 g/point was enough for pain stimulation.[19] In the comatose state, no animal reacted to 1 kg pressure.

Histopathological analysis

At 2 months after the whole surgery, the OB and pores of fila olfactoria sections were extracted, fixed with 0.9% formalin solution for 2 days, then embedded in paraffin blocks and cut into 5-µm thick sections using a microtome and stained with hematoxylin-eosin, glial fibrillary acidic protein immunofluorescence and terminal deoxynucleotidyl transferase dUTP nick end labeling dye staining for the examination of OBL-related alterations. Specimens were evaluated stereologically to measure fibrotic/occlusive variations in the pores of fila olfactoria per cubic centimeter and compared with OB volume values and sevoflurane induction times statistically.

The surface of olfactoria pores was estimated via the summation of each pore surface. Each pore surface was taken as an ellipsoid and surfaces were calculated. The surface value of each pore was estimated with the following formula:

$$\sum_1^n \mathrm{SOP} = \sum_{p=1}^{n} \mathrm{n}\pi r^2 \quad (1)$$

where SOP indicates total surface values of all olfactory pores of each animal; p indicates pores in the phyla olfactory; n indicates number of pores in phyla olfactory.

In the formula, the total surface area of n pores was calculated as $n\pi r^2$. Because olfactory pores are circle shaped, their surface area values were estimated as the πr^2.

The physical dissector method was used as in the study Aydin et al.[20] Two consecutive sections (dissector pairs) taken from reference tissue samples were mounted on each slide. The paired reference sections were reversed to double the number of dissector pairs without having to cut new sections. The mean numerical density of the olfactoria pores per/cm^2 fila olfactoria (Nt/Gt) per mm^2 was calculated using the following formula:

$$\frac{Nt}{Gt} = \frac{\sum Q^- N}{\sum A} / xd \quad (2)$$

where ΣQ–N is the total number of calculated pores found only in the reference sections, d is the section thickness, and A is the area of the counting frame. ΣA for the set of dissectors can be estimated from ΣA = ΣPa. ΣP is the total number of counting set frame points, and a is a constant area associated with a set point. The total number of pores in each specimen was obtained from the Cavalieri volume estimation method as in the studies of Aydin et al.[20,21]

Statistical analysis

All data are expressed as mean ± standard deviation. The SPSS® statistical software package version 21 (IBM Corp., Armonk, NY, USA) was used to compare the differences in the induction time of sevoflurane in the control, Sham, and OBL groups. The *P*-value was accepted as significant at the level of 0.05. Kruskal-Wallis test showed statistically significant differences among three groups.

Results

Histopathological findings

The total number of fila olfactoria pores was estimated as 3 ± 1 in all animals. Foramina did not close totally in animals subjected to OBL, but near-total obstruction of only soft tissues and not bone occurred due to fibrogliotic membrane-duro-arachnoid adhesions. The study group showed more obliterated or closed foramina than the control or sham groups. Meningeal inflammation, pia-arachnoid thickening, olfactory-fila losing, and nasal mucosal inflammatory bands were detected in the OBL group (**Figure 1**).

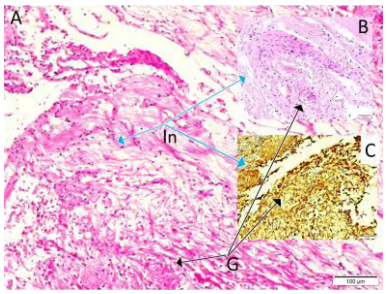

Figure 1: Histopathological view of an animal in the study group.
Note: Severely inflamed (In), deformed and degenerated olfactory glomerulus (G) is seen histopathologically in this figure. Thickened olfactory fila extensions (A), inflammation cells (B)), conjoined, deformed appendages of the fila olfactory, surrounded by inflammatory cells (C) (hematoxylin-eosin staining, original magnification 10×; immunofluorescence staining, original magnification 4×).

The gross anatomical appearances of OBs and histological appearance of olfactory fila and glomerulus of OBs in a control group rat areshown in **Figures 2** and **3**.

Quantitative results

The volume values of OBs and pore numbers significantly were diminished with the fibrosis of OBs in animals subjected to OBL ($P < 0.005$; **Table 1**). The induction time of sevoflurane was the longest in the study group, moderate in the sham group, and shortest in the control group ($P < 0.001$).

Discussion

This study indicates that rodents receiving OBL have a longer inhalation induction time than controls. Validity in research refers to how accurately a study answers the study question or the strength of the study conclusions.[22] Here, validity refers to how the induction time of sevoflurane changes after OBX. This is one of the first scientific explanations based on some ancient theories about how spiritual or tangible things could pass to the brain via the nasal route. The induction time was prolonged in rats with BL. This finding has clinical implications.

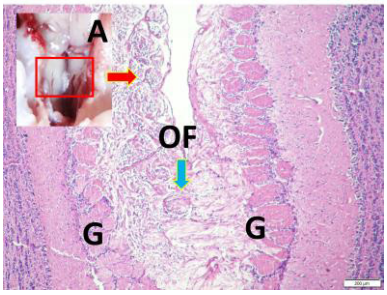

Figure 2: Gross anatomical appearances of lesioned OBs (A), destructed olfactory fila (OF), degenerated glomerulus (G) of OBs and apoptotic degeneration of OBs in an OBL rat (hematoxylin-eosin staining, original magnification 10×; terminal deoxynucleotidyl transferase dUTP nick end labeling dye staining, original magnification 10×).
Note: OB: olfactory bulb; OBL: olfactory bulb lesion.

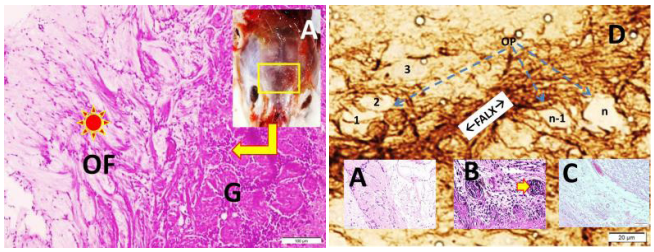

Figure 3: The pathological structure of OBs in control and study groups.
Note: Left: Gross anatomical appearances of OBs (A), olfactory fila (OF, arrow), and glomerulus (G) of OBs in the control group (hematoxylin-eosin staining, original magnification 10×, scale bar: 200 μm). Right: Gross anatomical appearances of lesioned OBs (A), destructed olfactory fila (OF, arrow), degenerated glomerulus (G) of OBs (hematoxylin-eosin staining, original magnification 10×, scale bar: 200 μm) and glial neuronal degeneration of OBs (marked with glial fibrillary acidic protein, original magnification 10×, scale bar: 200 μm) in OBL rat. (C) Gross anatomical appearances of lesioned OBs (A) under the burr hole defect illuminated cranium (yellow square) (A), destructed/regenerated olfactory fila (OF) surrounded by gliotic fibrotic materials (red star) and degenerated glomerulus (G) of OBs in OBL rat (hematoxylin-eosin staining, original magnification 10×, scale bar: 100 μm). (D) Histopathological appearances of partially-totally closed olfactory pores (OP; 1-n) among deformed olfactory files at the right-left sides of falx cerebri in a study animal (immunofluorescence staining, original magnification 40×). OB: olfactory bulb; OBL: olfactory bulb lesion.

Table 1: The number of fila olfactoria pores, and OB volume, and induction times

	Number of fila olfactoria pores	OB volume (mm^3)	Induction time (s)
Control group ($n = 9$)	10±3	4.51±0.99	124.44±26.17
Sham group ($n = 8$)	8±2	4.04±0.87	112.37±11.28
OBX group ($n = 12$)	5±1	2.34±0.42	164.33±30.92

Note: Data are expressed as mean ± SD and were analyzed by the Kruskal-Wallis test. OB: olfactory bulb; OBX: olfactory bulbectomy.

The "nose-to-brain delivery" seems to be a potential route for drugs targeting the brain. We investigated the effect of OBL on the induction time of sevoflurane, based on a hypothesis that the olfactory route would be a possible pathway for the entry of inhalational anesthetics, and found that the induction time of sevoflurane was increased in the OBL group compared with the control and Sham groups. Our results have clinical implications.

Certain anatomical structures in the skull base called for detailed study because of their functional importance,[23,24] such as OB which is a paired oval-shaped neural structure of the anterior cranium base. The cribriform plate provides a passage between the OB and the brain. Subarachnoid space around the fila olfactoria connected to the brain ventricles via neural foramina of the cribriform plates.

Sevoflurane exposure may induce neuronal cell changes in the brain by rapid equilibration between the alveolar and effect site (brain) concentrations, but the mechanism of action of sevoflurane is still not known. Though the exact mechanism of absorption of sevoflurane from the intra-nasal route is unknown, many established proven studies are showing perineural and perivascular paths around the olfactory and trigeminal nerve.[25] There is a question. What is the difference that sevoflurane is taken up by the olfactory mucosa and what is the evidence that OB injury may compromise that uptake? The main finding of this study is that the induction time of sevoflurane was increased in the OBL group. If we consider the nervous system as a great orchestra with complex harmonic combinations,[26] we will find it easier to understand how any OBL will be translated into an alteration of the effect of sevoflurane. All sensory information, except for the sense of smell, passes through the thalamus before processing by the cortex, so smell is the only one of the five senses, which is transmitted directly into cortical areas from the bulb without passing through the thalamus.[27] Interestingly, we found that damaged fila olfactoria could cause delayed induction time of sevoflurane. The explanation of this finding may be that inhalation anesthetics could move into the brain ventricles via fila olfactoria and show their effects.

Currently, medicine has gone through moments of great renewal,[28] despite these recent developments in technology,[29] the main effects of sevoflurane are still not well known; the established mechanism of pulmonary transport involves the movement of drugs across the alveolocapillary membrane into the blood and reaching the required terminal centers to induce the first anesthetic effect. The blood-brain and blood-cerebrospinal fluid barriers are important functional structures of the brain.[30] We hypothesized that sevoflurane could be absorbed from nasal cavities and respiratory tracts and transmitted into the blood circulation and contact surfaces. The drugs may show their effects after crossing the blood-brain barriers at required cerebrospinal fluid concentrations. To prove our hypothesis, OBs were crushed using a surgical clamp to create a closed fila olfactoria to prevent sevoflurane from passing into the brain. Sevoflurane has been in clinical use for inhalation anesthesia. Despite the detailed characterizations of the molecular and cellular pharmacology of sevoflurane, there is no study on whether volatile agents pass into the cerebrospinal fluid via fila olfactoria. So, we investigated whether there was any relationship between the induction time of inhalation anesthetics and open/closed conditions of fila olfactoria pores in OBL-performed animals, and found increased induction time of sevoflurane. Our finding could be attributed to the blocking of transport route by closed fila olfactoria in OBL performed animals. This is a novel finding that has not been reported previously. It was found an increased induction time in OBL rats and concluded that increased induction times of sevoflurane may be due to the OBL.

Several limitations of our study should be acknowledged. The main result of this study is that the induction time is increased in OBL rats, so the methodology of measuring the induction time accurately is necessary. It is not possible to analyze similar histopathological changes *in vivo* or through autopsy, because it is not possible to OBL in a man. There are some limitations to the present study. The dose of the anesthetic agents and induction phase measurement techniques, OBL application methods, and histological analysis of specimens were been done using currently available methods whereas superior modern techniques might be used. For example, anesthetic agents might be given with rat-specific anesthetic devices, and histopathological analyses might be done via electron microscopy. It may ask whether there is a sufficient amount of diffusion of sevoflurane at the nasal mucosa to cause neural effects except for smelling, considering big differences in the structure and the size of the surface area between the pulmonary alveolar membrane and the nasal mucosa. In this study, a single burr hole technique was used. In our studies of OBX, we think that one burr hole, rather than two, is sufficient to perform olfactory bulbus lesion. For that reason, we used one burr-hole as in our previous studies.[11,18] There is no other cause for using one burrhole. This technique should be compared to the "conventional technique" involving two frontal burr holes overlying the OBs. Also, it should be remembered that there are some differences in olfactory structure in rats. Rats seem to have a highly developed sense of smell compared to humans. These differences in the sense of smell between rats and humans can affect the results of the present study. The “higher value” of 165 seconds was arbitrarily accepted as “late induction time.” The relevance of a “late induction time” is prolonged induction time. As can be seen from the table, some changes in the Sham group were observed. In this study, we used an extremely traumatic injury that not only caused olfactory-fila loss but meningeal inflammation, and pia-arachnoid thickening may have occurred. Sevoflurane may have anti-inflammatory effects. Such changes in neuroinflammation may have caused unanticipated global changes in the brain that may have affected the results such as in the Sham group. Immunity is an important issue for the central nervous system.[31,32] The immunological impact of the anesthetic agents may lead to changes in the overall mortality and morbidity of patients. In the present study, we did not measure biomarkers of inflammation and oxidative stress. The disparity in the sample size of the control and sham groups and the experimental group may be another limitation. We know that the sample size is important in an experimental study. Equal sample sizes can give a greater power to detect differences. The intended sample size should be equal to the population.[33]

In conclusion, damage to the OB prolongs the induction time for sevoflurane, suggesting that its transport into the brain is impaired compared to control animals. We observed alveoli-capillary tight junction-like structures in fila olfactoria. Even though the induction phases of inhalation anesthetics were almost the same in all animals, the induction time was prolonged in the study group rats with OBL. histopathological analysis proved that tight junctions and fila olfactoria orifices were closed secondary to developed fibrogliotic membranes

over the cribriform plate in animals with OBL. That is, closed fila olfactoria orifices could not allow the transmission of inhalation anesthetics to the cerebrospinal fluids or the brain, and as a result, the tightly closed cribriform lamina orifices cause longer induction phases of inhalation anesthetics. The increased induction time of sevoflurane after OBL in rats is an important finding of this study which is reported for the first time, because the present study suggests that OBX blocks the olfactory transport of intranasally applied drugs to the central nervous system. More observational experimental studies are required to identify best practices, and understand disparities in access to and delivery of sevoflurona.

Author contributions

AA and MDA conceived and designed research. SO and HK conducted experiments. HK contributed nalytical tools. SO was responsible for data analysis. AK wrote the manuscript. All authors read and approved the final version of the manuscript.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Data availability statement

No additional data are available.

Open access statement

References

1. Ong Sio LCL, Dela Cruz RGC, Bautista AF. A comparison of renal responses to sevoflurane and isoflurane in patients undergoing donor nephrectomy: a randomized controlled trial. *Med Gas Res*. 2017;7:19-27.
2. Zuo Z. Are volatile anesthetics neuroprotective or neurotoxic? *Med Gas Res.* 2012;2:10.
3. Jiang M, Sun L, Feng DX, et al. Neuroprotection provided by isoflurane pre-conditioning and post-conditioning. *Med Gas Res*. 2017;7:48-55.
4. Blum FE, Zuo Z. Volatile anesthetics-induced neuroinflammatory and anti-inflammatory responses. *Med Gas Res.* 2013;3:16.
5. Liang TY, Peng SY, Ma M, Li HY, Wang Z, Chen G. Protective effects of sevoflurane in cerebral ischemia reperfusion injury: a narrative review. *Med Gas Res.* 2021;11:152-154.
6. Wetterkamp M, Meiser A, Weber TP, et al. Spontaneous breathing for managing analgesia during balanced anesthesia with remifentanil and desflurane: a prospective, single center randomized controlled trial. *Med Gas Res.* 2021;11:94-99.
7. Ozdemir B, Kanat A, Erturk C, et al. Important aspect of hypoglossal nerve injury following gunshot wound; Can the clivus has a role? A case report. *Br J Neurosurg*. 2020.
8. Batcik OE, Kanat A, Cankay TU, et al. COVID-19 infection produces subarachnoid hemorrhage; acting now to understand its cause: A short communication. *Clin Neurol Neurosurg.* 2021;202:106495.
9. Kanat A. Letter to the editor regarding "Aneurysmal subarachnoid hemorrhage in COVID-19 patients: a case series". *World Neurosurg.* 2021;154:199.
10. Karamali K, Elliott M, Hopkins C. COVID-19 related olfactory dysfunction. *Curr Opin Otolaryngol Head Neck Surg*. 2022;30:19-25.
11. Aydin MD, Kanat A, Hacimuftuoglu A, Ozmen S, Ahiskalioglu A, Kocak MN. A new experimental evidence that olfactory bulb lesion may be a causative factor for substantia nigra degeneration; preliminary study. *Int J Neurosci.* 2021;131:220-227.
12. Percie du Sert N, Hurst V, Ahluwalia A, et al. The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol*. 2020;18:e3000410.
13. Ruda-Kucerova J, Zanda MT, Amchova P, Fratta W, Fattore L. Sex and feeding status differently affect natural reward seeking behavior in olfactory bulbectomized rats. *Front Behav Neurosci.* 2018;12:255.
14. Kangralkar G, Jamale PB. Sevoflurane versus halothane for induction of anesthesia in pediatric and adult patients. *Med Gas Res.* 2021;11:53-57.
15. Figueira ERR, Rocha-Filho JA, Lanchotte C, et al. Sevoflurane preconditioning plus postconditioning decreases inflammatory response with hemodynamic recovery in experimental liver ischemia reperfusion. *Gastroenterol Res Pract.* 2019;2019:5758984.
16. Beck-Schimmer B, Baumann L, Restin T, et al. Sevoflurane attenuates systemic inflammation compared with propofol, but does not modulate neuro-inflammation: A laboratory rat study. *Eur J Anaesthesiol*. 2017;34:764-775.
17. Song Y, Chu R, Cao F, et al. Dopaminergic neurons in the ventral tegmental-prelimbic pathway promote the emergence of rats from sevoflurane anesthesia. *Neurosci Bull*. 2022;38:417-428.
18. Kanat A, Aydin MD, Akca N, Ozmen S. First histopathological bridging of the distance between Onuf's nucleus and substantia nigra after olfactory bulbectomy-new ideas about the urinary dysfunction in cerebral neurodegenerative disease: an experimental study. *Low Urin Tract Symptoms.* 2021;13:383-389.
19. Blivis D, Haspel G, Mannes PZ, O'Donovan MJ, Iadarola MJ. Identification of a novel spinal nociceptive-motor gate control for Aδ pain stimuli in rats. *Elife*. 2017;6:e23584.
20. Aydin MD, Kanat A, Yilmaz A, et al. The role of ischemic neurodegeneration of the nodose ganglia on cardiac arrest after subarachnoid hemorrhage: an experimental study. *Exp Neurol*. 2011;230:90-95.
21. Aydin MD, Kanat A, Yolas C, et al. Spinal subarachnoid hemorrhage induced intractable miotic pupil. a reminder of ciliospinal sympathetic center ischemia based miosis: an experimental study. *Turk Neurosurg*. 2019;29:434-439.
22. Ozdemir B, Kanat A, Durmaz S, Ersegun Batcik O, Gundogdu H. Introducing a new possible predisposing risk factor for odontoid type 2 fractures after cervical trauma; Ponticulus posticus anomaly of C1 vertebra. *J Clin Neurosci*. 2022;96:194-198.
23. Yilmaz A, Aydin MD, Kanat A, et al. The effect of choroidal artery vasospasm on choroid plexus injury in subarachnoid hemorrhage: experimental study. *Turk Neurosurg.* 2011;21:477-482.
24. Findik H, Kanat A, Celiker FB, et al. What are we missing from asymmetric relationship between the retinal nerve fiber layer thickness profiles and sphenoid sinus volume? *J Craniofac Surg*. 2020;31:e210-e214.
25. Lochhead JJ, Davis TP. Perivascular and perineural pathways involved in brain delivery and distribution of drugs after intranasal administration. *Pharmaceutics*. 2019;11:598.
26. Gasenzer ER, Kanat A, Neugebauer E. Neurosurgery and music; effect of Wolfgang Amadeus Mozart. *World Neurosurg.* 2017;102:313-319.
27. Yildiz I, Bayır H, Saglam I, et al. The effect of desflurane on postoperative olfactory memory. *Eur Rev Med Pharmacol Sci*. 2016;20:2163-2167.
28. Gasenzer ER, Kanat A, Ozdemir V, Neugebauer E. Analyzing of dark past and bright present of neurosurgical history with a picture of musicians. *Br J Neurosurg.* 2018;32:303-304.
29. Kanat A, Balik MS, Kirbas S, et al. Paradox in the cubital tunnel syndrome--frequent involvement of left elbow: first report. *Acta Neurochir (Wien).* 2014;156:165-168.
30. Kanat A. Risk factors for neurosurgical site infections after craniotomy: a prospective multicenter study of 2944 patients. *Neurosurgery.* 1998;43:189-190.
31. Aydin MD, Kanat A, Yolas C, Calik M. New insights of the fever following subarachnoid hemorrhage and introducing a new thermoregulator like structure in choroid plexuses; preliminary study. *Acta Neurol Taiwan.* 2019;28(1):1-11.
32. Aydın MD, Kanat A, Aydın N, et al. New evidence for causal central mechanism of hyperglycemia in subarachnoid hemorrhage secondary to ischemic degenerative disruption of circuitry among insular cortex, nodose ganglion, and pancreas: experimental study. *World Neurosurg.* 2017;106:570-577.
33. Caglar O, Firinci B, Aydin ME, et al. First emerging evidence of the relationship between Onuf's nucleus degeneration and reduced sperm number following spinal subarachnoid haemorrhage: Experimental study. *Andrologia*. 2021;53:e14030.

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